External Review Draft
EPA/635/R-22/039b
www.epa.gov/iris

Toxicological Review of Formaldehyde—Inhalation Supplemental Information

[CASRN 50-00-0]

April 2022

Integrated Risk Information System
Center for Public Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

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ABBREVIATIONS

α2u	alpha 2u-globulin
ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike's information criterion
ALD	approximate lethal dosage
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service Registry Number
CBI	covalent binding index
CHO	Chinese hamster ovary (cell line cells)
CL	confidence limit
CNS	central nervous system
CPN	chronic progressive nephropathy
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DEN	diethylnitrosamine
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GSH	glutathione
GST	glutathione-S-transferase
Hb/g-A	animal blood:gas partition coefficient
Hb/g-H	human blood:gas partition coefficient
HEC	human equivalent concentration
HED	human equivalent dose
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IVF	in vitro fertilization
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocyte
MTD	maximum tolerated dose
NAG	N-acetyl-β-D-glucosaminidase
NCEA	National Center for Environmental Assessment
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand White (rabbit breed)
OCT	ornithine carbamoyl transferase
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
PCNA	proliferating cell nuclear antigen
POD	point of departure
$POD_{[ADJ]}$	duration-adjusted POD
QSAR	quantitative structure-activity relationship
RDS	replicative DNA synthesis
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
RNA	ribonucleic acid
SAR	structure activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SGOT	glutamic oxaloacetic transaminase, also known as AST
SGPT	glutamic pyruvic transaminase, also known as ALT
SSD	systemic scleroderma
TCA	trichloroacetic acid
TWA	time-weighted average
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_H	intraspecies uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
UF_D	database deficiencies uncertainty factor

APPENDIX A. INFORMATION IN SUPPORT OF HAZARD IDENTIFICATION

A.1. CHEMICAL PROPERTIES AND HUMAN EXPOSURE

A.1.1. Chemical Properties

Formaldehyde (CASRN 50-00-0) is the first of the series of aliphatic aldehydes and is a gas at room temperature. Its molecular structure is depicted in Figure A-1. It is noted for its reactivity and versatility as a chemical intermediate. It readily undergoes polymerization, is highly flammable, and can form explosive mixtures with air. It decomposes at temperatures above 150°C (WHO, 2002).

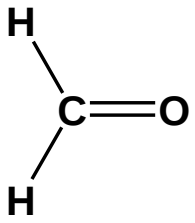

Figure A-1. Chemical structure of formaldehyde.

At room temperature, pure formaldehyde is a colorless gas with a strong, pungent, suffocating, and highly irritating odor (NLM, 2019). Formaldehyde is readily soluble in water, alcohols, ether, and other polar solvents (WHO, 2002). A synopsis of its physicochemical properties is given in Table A-1.

Production, uses, and sources of formaldehyde

Formaldehyde has both commercial and industrial uses. Formaldehyde has been produced commercially since the early 1900s and, in recent years, has been ranked in the top 25 highest volume chemicals produced in the U.S. (NTP, 2010; ATSDR, 1999). Based on EPA's Chemical Data Reporting (CDR) the national production volume for formaldehyde was 3.9 billion lb/yr in 2011 and between 1 and 5 billion lbs/yr for the years 2012 through 2015 (https://chemview.epa.gov/chemview/#).

Table A-1. Physicochemical properties of formaldehyde

Name	Formaldehyde
International Union for Pure and Applied Chemistry name	Formaldehyde
Synonyms	Formic aldehyde Methanal Methyl aldehyde Methylene oxide Oxomethane Oxymethylene
Chemical Abstracts Service Index name	Formaldehyde
Chemical Abstracts Service Registry Number	50-00-0
Formula	HCHO
Molecular weight	30.03
Density	Gas: 1.067 (air = 1) Liquid: 0.815 g/mL at −20°C
Vapor pressure	3,883 mm Hg at 25°C
Log K_{ow}	−0.75 to 0.35
Henry's law constant	3.4×10^{-7} atm-m^3/mol at 25°C 2.2×10^{-2} Pa-m^3/mol at 25°C
Conversion factors (25°C, 760 mm Hg)	1 ppm = 1.23 mg/m^3 (v/v) 1 mg/m^3 = 0.81 ppm (v/v)
Boiling point	−19.5°C at 760 mm Hg
Melting point	−92°C
Flash point	60°C; 83°C, closed cup for 37%, methanol-free aqueous solution; 50°C closed cup for 37% aqueous solution with 15% methanol
Explosive limits	73% upper; 7% lower by volume in air
Autoignition temperature	300°C
Solubility	Very soluble in water; soluble in alcohols, ether, acetone, benzene
Reactivity	Reacts with alkalis, acids and oxidizers

Sources: Gerberich and Seaman (2013); WHO (2002); ACGIH (2001); ATSDR (1999); Walker (1975)

Approximately 55% of the consumption of formaldehyde is in the production of industrial resins (NTP, 2010). Formaldehyde is a chemical intermediate used in the production of some plywood adhesives, abrasive materials, insulation, foundry binders, brake linings made from phenolic resins, surface coatings, molding compounds, laminates, wood adhesives made from melamine resins, phenolic thermosetting, resin curing agents, explosives made from hexamethylenetetramine, urethanes, lubricants, alkyd resins, acrylates made from trimethylolpropane, plumbing components from polyacetal resins, and controlled-release fertilizers made from urea formaldehyde concentrates (IPCS, 1989), as cited in (ATSDR, 1999). Formaldehyde is used in smaller quantities for the preservation and embalming of biological specimens. It is also used as a germicide, an insecticide, and a fungicide in some products. It is found (as an ingredient or impurity) in some cosmetics and personal hygiene products, such as some soaps, shampoos, hair preparations, deodorants, sunscreens, dry skin lotions, and mouthwashes, mascara and other eye

makeup, cuticle softeners, nail creams, vaginal deodorants, and shaving cream (NTP, 2010; WHO, 2002; ATSDR, 1999).

Formaldehyde is commonly produced as an aqueous solution called formalin, which is used in industrial processes and usually contains about 37% formaldehyde and 12–15% methanol. Methanol is added to formalin to slow polymerization that leads eventually to precipitation as paraformaldehyde. Paraformaldehyde has the formula $(CH_2O)_n$, where n is 8 to 100. It is essentially a solid form of formaldehyde and therefore has some of the same uses as formaldehyde (Kiernan, 2000). When heated, paraformaldehyde sublimes as formaldehyde gas. This characteristic makes it useful as a fumigant, disinfectant, and fungicide, such as for the decontamination of laboratories, agricultural premises, and barbering equipment. Long-chain polymers (e.g., Delrin plastic) are less inclined to release formaldehyde, but they have a formaldehyde odor and require additives to prevent decomposition.

The major sources of anthropogenic emissions of formaldehyde are motor vehicles, power plants, manufacturing plants that produce or use formaldehyde or substances that contain formaldehyde (i.e., adhesives), petroleum refineries, coking operations, incineration, wood burning, and tobacco smoke. Among these anthropogenic sources, the greatest volume source of formaldehyde is automotive exhaust from engines not fitted with catalytic converters (NEG, 2003). The Toxic Release Inventory (TRI) data for 2016 show total releases of 19.4 million pounds with about 13 million to underground injection (EPA TRI Explorer, https://enviro.epa.gov/triexplorer/tri_release.chemical).

Formaldehyde is formed in the lower atmosphere by photochemical oxidation of hydrocarbons or other formaldehyde precursors that are released from combustion processes (ATSDR, 1999). Formaldehyde can also be formed by a variety of other natural processes, such as decomposition of plant residues in the soil, photochemical processes in sea water, and forest fires (NLM, 2019).

The input of formaldehyde into the environment is counterbalanced by its removal by several pathways. Formaldehyde is removed from the air by direct photolysis and oxidation by photochemically produced hydroxyl and nitrate radicals. Measured or estimated half-lives for formaldehyde in the atmosphere range from 1.6 to 19 hours, depending upon estimates of radiant energy, the presence and concentrations of other pollutants, and other factors (ATSDR, 1999). Given the generally short daytime residence times for formaldehyde, there is limited potential for long-range transport (WHO, 2002). In cases where organic precursors are transported long distances, however, secondary formation of formaldehyde may occur far from the anthropogenic sources of the precursors.

Formaldehyde is released to water from the discharges of both treated and untreated industrial wastewater from its production and from its use in the manufacture of formaldehyde-containing resins (ATSDR, 1999). Formaldehyde is also a possible by-product from using ozone

and/or hydrogen peroxide for drinking-water disinfection. In water, formaldehyde is rapidly hydrated to form a glycol, and the equilibrium favors the glycol.

A.1.2. Human Exposure

While exposure assessments are not included in IRIS toxicological reviews, this section on human exposure to formaldehyde is intended to provide context for the analyses of hazard identification and dose-response presented in this assessment. General population exposure to formaldehyde can occur via inhalation, ingestion and dermal contact, with inhalation exposure representing the primary exposure route. Each of these pathways and associated media levels are discussed below. Formaldehyde exposure can occur occupationally via three main scenarios:

- The production of aqueous solutions of formaldehyde (formalin) and their use in the chemical industry (e.g., for the synthesis of various resins, as a preservative in medical laboratories and embalming fluids, and as a disinfectant).
- Release from formaldehyde-based resins in which it is present as a residue and/or through their hydrolysis and decomposition by heat (e.g., during the manufacture of wood products, textiles, synthetic vitreous insulation products, and plastics). In general, the use of phenol-formaldehyde resins results in much lower emissions of formaldehyde than those of urea- based resins.
- The pyrolysis or combustion of organic matter (e.g., in engine exhaust gases or during firefighting) (IARC, 2006).

Occupational exposures occur not only during the production of products containing formaldehyde, but also during the use of these products in construction and decoration (Kim et al., 2011). Industries with the greatest potential for exposure include health services, business services, printing and publishing, manufacture of chemicals and allied products, manufacture of apparel and allied products, manufacture of paper and allied products, personal services, machinery (except clerical), transport equipment, and furniture and fixtures (IARC, 1995). Exposure levels for the workers of various professions in a selected number of studies range from 49 to 4,280 μg/m^3 (40 to 3,480 ppb), with plywood particle board production workers having the highest exposures (Kim et al., 2011).

In recent years, concerns have been raised regarding occupational exposures resulting from the use semi-permanent professional hair straightening products. In 2010, responding to requests from hair salon employees to the National Institute of Occupational Safety and Health (NIOSH), a study of hair smoothing treatment products marketed as formaldehyde free was conducted. The CDC study (2011) found that the formaldehyde content in a total of 105 samples of these products ranged from 6.8 to 11.8%, with an average of 8.8%. Air samples taken in seven hair salons during smoothing treatments showed 8-hour time-weighted average concentrations of formaldehyde ranging from 7.4 μg/m^3 (6 ppb) to 407.1 μg/m^3 (331 ppb) (CDC, 2011). Air concentrations vary depending on factors such as room ventilation, ceiling height, room size, and duration of the

treatment (CDC, 2011). Another study by Pierce et al. (2011) collected air samples during the use of four commercially available hair smoothing products. The hair stylist 8-hour time-weighted average concentrations of formaldehyde ranged from 24.6 μg/m^3 (20 ppb) to 196.8 μg/m^3 (160 ppb) for one treatment per day and 61.5 μg/m^3 (50 ppb) to 922.5 μg/m^3 (750 ppb) for four consecutive treatments (Pierce et al., 2011). Time weighted average concentrations decreased as the distance from the treatment location increased (Pierce et al., 2011).

Inhalation

EPA's AirToxScreen (https://www.epa.gov/AirToxScreen; note: a previous version was the National Air Toxics Assessment) provides modeled formaldehyde concentrations based on emissions inventories and meteorological data for areas such as counties, states and the nation and includes the contiguous US, Alaska, Hawaii, Puerto Rico, and Virgin Islands. The range of estimated county mean outdoor air concentrations is 0.1 – 4.3 μg/m^3. The breakout by Sector is illustrated in Figure A-2.

Ambient air monitoring data for formaldehyde are available from EPA's Ambient Monitoring Archive for HAPs which includes data from the Air Quality System database and other data sources (https://www.epa.gov/amtic/amtic-air-toxics-data-ambient-monitoring-archive). Measurement data are collected from National Air Toxic Trends Sites (NATTS) and other sites across the country operated by state, local, and tribal agencies that are not part of the NATTS network. Data for the year 2018, come from 100 monitors located in 27 states and the District of Columbia. The annual means for these monitors range from 0.25–11.06 μg/m^3 (0.20–9.01 ppb) and have an overall average of 2.97 μg/m^3 (2.42 ppb). The annual means were derived by EPA through averaging all available daily data from each site that has at least three valid quarters for the year (i.e., a valid quarter is a quarter that contains at least seven daily averages) (https://www.epa.gov/system/files/documents/2021-08/annual-average-statistics-documentation-2018.pdf). Table A-2 presents the data by land use category based on the annual means from each site for 2018. The land use is established in the Air Quality System database from the site description.

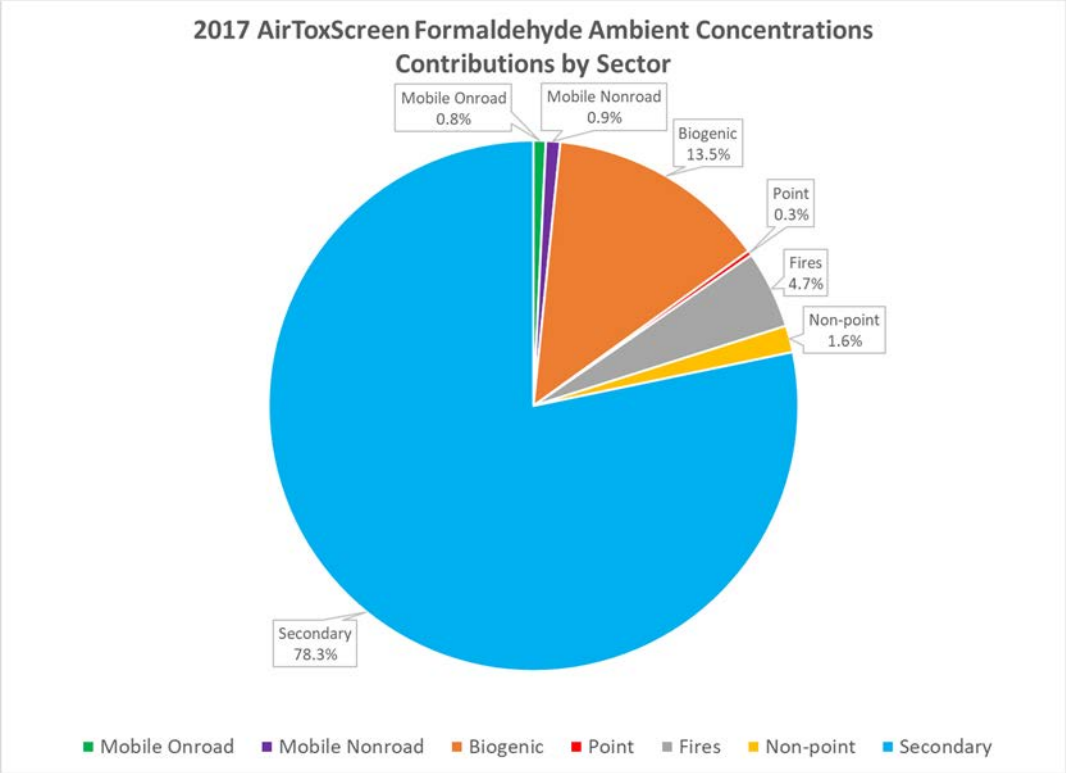

Figure A-2. Formaldehyde Ambient Concentrations Contribution by Sector.

Source: Based on 2017 AirToxScreen (EPA/OAR).

Table A-2. Ambient air levels by land use category based on 2018 annual site averages

	Annual formaldehyde ambient air concentrations by category (µg/m^3)					
	Agriculture	**Commercial**	**Forest**	**Industrial**	**Mobile**	**Residential**
Number of annual averages	5	31	4	11	6	43
Mean	2.02	2.88	1.98	3.42	3.80	3.00
Minimum	1.40	0.25	1.03	1.74	2.02	0.88
Maximum	2.61	4.84	3.40	8.25	5.71	11.06

Source: EPA's Ambient Monitoring Archive for HAPs which includes data from the Air Quality System and other data sources at https://www.epa.gov/amtic/amtic-air-toxics-data-ambient-monitoring-archive.

In general, ambient levels of formaldehyde in outdoor air are significantly lower than those measured in the indoor air of workplaces or residences (ATSDR, 1999; IARC, 1995). Indoor sources of formaldehyde in air include volatilization from pressed wood products, carpets, fabrics, insulation, permanent press clothing, latex paint, and paper bags, along with emissions from gas

burners, kerosene heaters, and cigarettes. Kim et al. (2015b) suggested that air fresheners, scented candles, and electric diffusers may also contribute to indoor concentrations of formaldehyde. Indoor air levels are affected by the age of the source materials, temperature, humidity, and ventilation rates (Parthasarathy et al., 2011; (IARC), 2006). Release rates of formaldehyde from consumer products have been published in the literature. Table A-3 presents a selected number of products and their respective emission rates in $\mu g/m^2$-hr.

In general, the major indoor air sources of formaldehyde can be described in two ways: (1) those sources that have the highest emissions when the product is new with decreasing emission over time, as with the first set in the examples above; and (2) those sources that are reoccurring or frequent such as the second set of examples above. Several studies were found in the literature that investigated indoor air concentrations of formaldehyde in various housing types. Median indoor air concentrations in various European countries in both commercial and residential buildings ranged from 10 $\mu g/m^3$ to 50 $\mu g/m^3$ (Sarigiannis et al., 2011). A summary of residential indoor air data in the U.S. and Canada is provided in Table A-4. These are organized by manufactured (i.e., mobile homes/trailers with wheels that are designed to be moved) and conventional housing and in chronological order, beginning with the most recent studies. Results vary depending on housing characteristics and date of study. In general, higher concentrations are found in manufactured houses.

Even though formaldehyde levels in construction materials have declined, indoor inhalation concerns still persist. For example, as shown in Table A-4, studies have measured formaldehyde levels in manufactured homes. ATSDR (2007) reported on air sampling in 96 unoccupied trailers provided by the Federal Emergency Management Agency (FEMA) used as temporary housing for people displaced by Hurricane Katrina (see Table A-4). Formaldehyde levels in closed trailers averaged 1,279 ± 849 $\mu g/m^3$ (mean ± standard deviation [SD]) (1.04 ± 0.69 ppm), with a range of 12–4,500 $\mu g/m^3$ (0.01–3.66 ppm). The levels decreased to an average of 480 ± 324 $\mu g/m^3$ (0.39 ± 0.27 ppm), with a range of 0.00–2,005 $\mu g/m^3$ (0.00–1.63 ppm) when the air conditioning was turned on. Levels also decreased to an average of 111 ± 98 $\mu g/m^3$ (0.09 ± 0.08 ppm), with a range of 12–603 $\mu g/m^3$ (0.01–0.49 ppm) when the windows were opened. ATSDR (2007) found an association between temperature and formaldehyde levels; higher temperatures were associated with higher formaldehyde levels in trailers with the windows closed. They also noted that different commercial brands of trailers yielded different formaldehyde levels.

In December 2007 and January 2008, the Centers for Disease Control and Prevention (CDC) measured formaldehyde levels in a stratified random sample of 519 FEMA-supplied occupied travel trailers, park models, and mobile homes ("trailers") (CDC, 2008). At the time of the study, sampled trailers were in use as temporary shelters for Louisiana and Mississippi residents displaced by hurricanes Katrina and Rita. The geometric mean level of formaldehyde in sampled trailers was 95 $\mu g/m^3$ (77 ppb), and the range was 3.7–726 $\mu g/m^3$ (3–590 ppb) (see Table A-4).

Another study by Maddalena et al. (2008) measured indoor air concentrations for a range of volatile organic compounds (VOCs), including formaldehyde in four unoccupied temporary housing units (i.e., mobile homes) under steady state ventilation conditions. A morning and afternoon measurements were taken for each unit. The overall average air concentration of formaldehyde for the four mobile homes was 569 μg/m^3. This is consistent with values measured by ATSDR (2007) and CDC (2008). Consistently higher air concentrations of formaldehyde were measured in the afternoon samples.

Air concentrations of formaldehyde were lower for conventional housing as shown in Table A-4. Mean values from studies published between 1980 and 2008 ranged from 6.2 to >1,230 μg/m^3. Although no conclusions could be drawn based on the age of the study alone, some of the studies in Table A-4 suggests that air concentrations are influenced by the age of the house and season of the year. Lower air concentrations were observed as the age of the house increased. Higher concentrations were generally observed during the summer months.

Salthammer et al. (2010) present a thorough review of formaldehyde sources and levels found in the indoor environment. Based on an examination of international studies carried out in 2005 or later they conclude that the average exposure of the population to formaldehyde is 20 to 40 μg/m^3 under normal living conditions. Figure A-3 summarizes the range of formaldehyde air concentrations in various environments. The dotted line represents the WHO guidelines of 100 μg/m^3. More recently, Branco et al. (2015) measured hourly mean formaldehyde concentrations as high as 204 μg/m^3 in nursery schools in Portugal.

Data on formaldehyde levels in outdoor and indoor air were collected under Canada's National Air Pollution Surveillance program (WHO, 2002; Health Canada, 2001). The effort included four suburban and four urban sites sampled in the period 1990–1998. A Monte Carlo analysis applied to the pooled data (n = 151) was used to estimate the distribution of time-weighted 24-hour air exposures. This study suggested that mean levels in outdoor air were 3.3 μg/m^3 (2.7 ppb) and mean levels in indoor air were 35.9 μg/m^3 (29.2 ppb) (Health Canada, 2001). The simulation analysis also suggested that general population exposures averaged 33–36 μg/m^3 (27–30 ppb).

Since the early to mid 1980s, manufacturing processes and construction practices have been changed to reduce levels of indoor formaldehyde emissions (ATSDR, 1999). A 2008 law enacted by the California Air Resource Board (Final Regulation Order: Airborne Toxic Control Measure to Reduce Formaldehyde Emissions from Composite Wood Products; http://www.arb.ca.gov/regact/2007/compwood07/fro-final.pdf) has limited the amount of formaldehyde that can be released by specific composite wood products (i.e., hardwood plywood, particle board, and medium density fiberboard) sold, supplied, or manufactured for use in California. For this reason, the mean indoor air levels presented by Health Canada (2001) (based on samples collected from 1989–1995) may overestimate current levels.

Table A-3. Formaldehyde emission rates from various consumer products

Products	Emission Rate (µg/m²-hr)	Reference
Pressed wood products	ND–1,500	Pickrell et al. (1983)
New clothing	0.63–31.25	Pickrell et al. (1983)
Insulation products	2.17–25.83	Pickrell et al. (1983)
Paper plates and cups	3.13–41.67	Pickrell et al. (1983)
Fabrics	ND–14.58	Pickrell et al. (1983)
Carpets	ND–2.71	Pickrell et al. (1983)
Carpets with urethane foam backing	411–6[a]	Yu and Crump (1998)
Textile carpet	83–36[a]	Yu and Crump (1998)
Carpet with synthetic/PVC fibers	120–11[a]	Yu and Crump (1998)
Carpet assembly	153,000–783[a]	Yu and Crump (1998)
Carpet underlay	8,110–12[a]	Yu and Crump (1998)
Vinyl/PVC flooring	22,280–91[a]	Yu and Crump (1998)
Linoleum flooring	220–22[a]	Yu and Crump (1998)
Vinyl tiles	91–45[a]	Yu and Crump (1998)
Rubber floorings	1,400[b]	Yu and Crump (1998)
Soft plastic flooring	590[b]	Yu and Crump (1998)
Cork floor tiles	805–7[a]	Yu and Crump (1998)
Mineral wool insulation batt	15–12[b]	Yu and Crump (1998)
Glass wool fibrous insulation	4–0.08	Yu and Crump (1998)
Extruded polystyrene thermal insulants	1,400–22[a]	Yu and Crump (1998)
Extruded polyethylene duct and pipe insulants	0.8–0.28[b]	Yu and Crump (1998)
Plastic laminated board	0.4[b]	Yu and Crump (1998)
Vinyl and fiber glass wallpaper	300[b]	Yu and Crump (1998)
PVC foam wallpaper	230	Yu and Crump (1998)
PVC wall covering	100	Yu and Crump (1998)
Vinyl coated wallpaper	95–20	Yu and Crump (1998)
Vinyl wallpaper	40	Yu and Crump (1998)
Wallpaper	100–31	Yu and Crump (1998)
Vapor barriers (bituminous tar)	6.3[c]	Yu and Crump (1998)
Black rubber trim for jointing	103	Yu and Crump (1998)
Vinyl covering	46–30[d]	Yu and Crump (1998)
Textile wall and floor coverings	1,600[b]	Yu and Crump (1998)
Acoustic partitions	158–6[a]	Yu and Crump (1998)
Office chair	1,060–100[a]	Yu and Crump (1998)
Particle board	1,500–2,167[e] 200–28[a]	Pickrell et al. (1984) Yu (Yu and Crump, 1998)
Plywood	1,292–1,375[e] 1,450–44	Pickrell et al. (1984) Yu and Crump (1998)
Bare urea-formaldehyde wood products (¼– ¾")	8.6–1,580[f]	Kelly et al. (1999)

Products	Emission Rate (µg/m²-hr)	Reference
Coated urea-formaldehyde wood products	<2.7–460[f]	Kelly et al. (1999)
Permanent press fabric	42–215[f]	Kelly et al. (1999)
Decorative laminates	4.2–51[f]	Kelly et al. (1999)
Fiberglass products	16–32[f]	Kelly et al. (1999)
Bare phenol-formaldehyde wood products	4.1–9.2[f]	Kelly et al. (1999)
Paper grocery bags	<0.5[f]	Kelly et al. (1999)
Paper towels	<0.6[f]	Kelly et al. (1999)
Latex paint	326–854[b]	Kelly et al. (1999)
Finger nail hardener	178,000–215,500[b]	Kelly et al. (1999)
Nail polish	20,700[b]	Kelly et al. (1999)
Commercially applied urea-formaldehyde floor finish	421–1,050,000[b]	Kelly et al. (1999)

[a] The first number in the range indicates initial emissions; the second number indicates emissions after some time (e.g., hours, days, months).
[b] Values represent initial emissions.
[c] 124 days old.
[d] <98 days old.
[e] Range indicates different test conditions in temperature and relative humidity.
[f] Emission rates represent typical conditions, defined as 70 °F, 50% Relative Humidity, and 1 air change per hour.

Table A-4. Studies on residential indoor air levels of formaldehyde

Location (year measured)	Na	Concentration mean (range); µg/m³	Reference
Manufactured housing			
LA & MS, FEMA-supplied temporary housing units (Dec. 2007–Jan. 2008)	519[b]	95 (3.7–726)[c]	CDC (2008)
FEMA 4 temporary housing units (2007)	4[b]	569 (331–926)	Maddalena et al. (2008)
Baton Rouge, LA, 96 FEMA-supplied temporary housing units (2006) Baseline[d] Ventilation with air conditioning and bathroom vents only Ventilation with open windows and vents	 96 852 863	 1,279 (12–4,500) 480 (0–2,005) 111 (12–603)	ATSDR (2007)
Florida, new manufactured house (2000)	NR	95 (NR)	Hodgson et al. (2002)[e]
United States, East and Southeast (1997–98)	4	42 (26–58)	Hodgson et al. (2000)[e]
California, mobile homes (1984–85)	470	86–111(NR)	Sexton et al. (1989)[f]
United States (NR) Complaint mobile homes Newer mobile homes Older mobile homes	 >500 260	 123–1,107 (0–5,166) 1,032 308	Gammage and Hawthorne (1985)
Texas, mobile homes whose residents requested testing (1979–82)	443[b]	NR (ND–9,840)	Norsted et al. (1985)[f]

Location (year measured)	Na	Concentration mean (range); µg/m³	Reference
Homes < 1 yr old Homes > 1 yr old		≥ 2,460 for 27% of homes ≥ 2,460 for 11.5% of homes	
United States (NR)	430[b]	> 1,230 for 4% of samples 615–1,230 for 18% of samples 123–615 for 64% of samples < 123 for 14% of samples	Breysse (1984)[g]
United States (NR)	431[b]	470 (12–3,599)	Ulsamer et al. (1982)[g]
United States (NR) Complaint homes, WA, < 2 yr old Complaint homes, WA, 2–10 yr old Complaint homes, MN, < 2 yr old Complaint homes, MN, 2–10 yr old Complaint homes, WI, < 2 yr old Complaint homes, WI, 2–7 yr old Random sample, WI, < 2 yr old	 110[b] 77[b] 66[b] 43[b] 38[b] 9[b] NR	 950 (NR) 581 (NR) 1,041 (NR) 339 (NR) 891 (NR) 560 (NR) 661 (NR)	Stone et al., 1981[g]
Wisconsin, complaint homes, 0.2–12 yr old (NR)	65[b]	590[h] (NR)	Dally et al. (1981)[g]
Conventional housing or unspecified			
California (2011-2013)	352[b]	21 (NR)	Vardoulakis et al. (2020)
Cincinnati, Ohio (2011) (median, IQR) Low income homes, renovated and nonrenovated, all measurements	96	20 (14—33)	Coombs et al. (2016)
Quebec City, Canada (2008-2011)	83[b]	37 (NR)	Vardoulakis et al. (2020)
Summer Field, CA (2006)	52[b]	36 (4.7–143.6)	Offermann et al. (2008)
Québec, Canada (2005)	96[b]	30 (9.6–90)	Gilbert et al. (2006)
Prince Edward Island, Canada (winter 2002)	59[b]	39.0 (5.5–87.5)	Gilbert et al. (2005)
Los Angeles, CA; Houston, TX, and Elizabeth, NJ (summer 1999–spring 2001)	398	22 ± 7.1[i]	Weisel et al. (2005)
New York City, NY(46 houses)(1999), Los Angeles, CA (41 houses) (2000) NYC (winter) NYC (summer) LA (winter) LA (fall)	 37 41 40 33	 12 ± 4.7 (5.2–22) 21 ± 11 (5.8–51) 21 ± 11 (7.9–59) 16 ± 6.2 (8.2–32)	Sax et al. (2004)
Canada (1989–1995) Northwest Territories; Windsor, Ontario; Hamilton, Ontario; Trois-Rivières, Québec; Saskatoon, Saskatchewan	151	36 (12–144)	Environment Canada (2000)
United States, East and Southeast, site-built houses (1997–1998)	7	44[j] (17–71)	Hodgson et al. (2000)[e]
Arizona (Jun. 1995–Feb. 1998)	189	21[h] (max. 408)	Graf et al. (1999)

Location (year measured)	Na	Concentration mean (range); µg/m³	Reference
Louisiana, 53 houses: 75% urban;25% rural (NR)	419	460 (ND–6,599)	Lemus et al. (1998)[e]
Boston, MA (1993) winter, 4 residences summer, 9 residences	14 26	13.7 (7.4–19.8) 19.8 (7.3–66.2)	Reiss et al. (1995)[e]
Maryland (1995) Newly build house 30 days after installation pressed wood	1[b]	 <94 55	Hare et al. (1996)
Colorado (1992–93) Prior to occupancy After occupancy for 5 months	9	 26 (8.0–66) 49 (33.0–81.2)	Lindstrom et al. (1995)[e]
New Jersey, 6 residential houses (1992)	36	67.1 (33–125)	Zhang et al. (1994)
Arizona, houses (NR)	202[b]	32 (max. 172)	Krzyzanowski et al. (1990)[d]
United States, residential, various locations (1981–84)	273	44.0[h](NR)	Shah and Singh (1988)[b]
San Francisco, CA, Bay Area (1984) Kitchen Main bedroom	 48 45	 50 (NR) 44 (NR)	Sexton et al. (1986)[b]
United States (NR) Homes with UFFI Homes with UFFI	 >1,200 131	 62–148 (123–4,182) 31–86 (12–209)	Gammage and Hawthorne (1985)
Pullman, WA, houses (NR)	NR	6.2–89 (NR)	Lamb et al. (1985)[f]
United States (NR) UFFI houses Non-UFFI houses and apartments	 244[b] 59[b]	 > 1,230 for 2.8% of samples 615–1,230 for 1.9% of samples 123–615 for 24.1% of samples < 123 for 71.2% of samples > 1,230 for 1.8% of samples 615–1,230 for 1.8% of samples 123–615 for 36.3% of samples < 123 for 60.1% of samples	Breysse (1984)[g]
United States (1982) Houses 0–30 yr old Houses 0–5 yr old Houses 5–15 yr old Houses > 15 yr old Houses 0–5 yr old spring summer autumn Houses 5–15 yr old spring summer autumn	 40[b] 18[b] 11[b] 11[b] 18[b] 11[b] 	 75.9 ± 95.0[i] 103.0 ± 112.1[i] 52.0 ± 52.0[i] 39.0 ± 52.0[i] 107.0 ± 114.0[i] 137 ± 125[i] 58.0 ± 68.0[i] 53.0 ± 49.0[i] 60.0 ± 59.0[i] 41.9 ± 43.1[i]	Hawthorne et al. (1983)[g]

Location (year measured)	Na	Concentration mean (range); µg/m³	Reference
Houses > 15 yr old			
spring	11[b]	44.0 ± 63.0[i]	
summer		36.0 ± 46.0[i]	
autumn		32.0 ± 28.0[i]	
United States (1983)			Grimsrud et al. (1983)[g]
Energy-efficient new houses	20[b]	76 (NR)	
Low-ventilation modernized houses	16[b]	37 (NR)	
United States (1981)			Ulsamer et al. (1982)[g]
Houses without UFFI	41[b]	40 (12–98)	
Houses with UFFI	636[b]	150 (12–4,200)	
United States (1980–81)			Offerman et al., 1982[g]
Houses averaging 2 yr old	9[b]		
air-tight construction		44 ± 22[i]	
mechanical ventilation		33 ± 20[i]	
Houses averaging 6 yr old (loose construction)	1[b]	17 (NR)	
United States (1978–79)	13[b]	120[h](NR)	Dally et al. (1981)[g]
United States (1979)	2[b]		Berk et al. (1980)[g]
Energy-efficient house		98 (40–150)	
Unoccupied house without furniture		81 ± 7.0[i]	
Unoccupied house with furniture		225 ± 16.0[i]	
Occupied house			
day		263 ± 26.0[i]	
night		141 ± 44.0[i]	

Note: Concentrations were converted from ppb to µg/m³ for consistency (1 ppb = 1.23 µg/m³).
ND = not detected; NR = not reported.
[a] Number of samples unless denoted with footnote (b).
[b] Number of houses.
[c] Geometric mean.
[d] Baseline refers to initial levels measured 4 days prior to intervention phase of the study during which ventilation via air conditioning or open windows was provided.
[e] Cited in (IARC) (2006).
[f] Cited in ATSDR (1999).
[g] Cited in IPCS (1989).
[h] Median.
[i] Standard deviation.
Source: Adapted from NTP (2010) and other sources as noted.

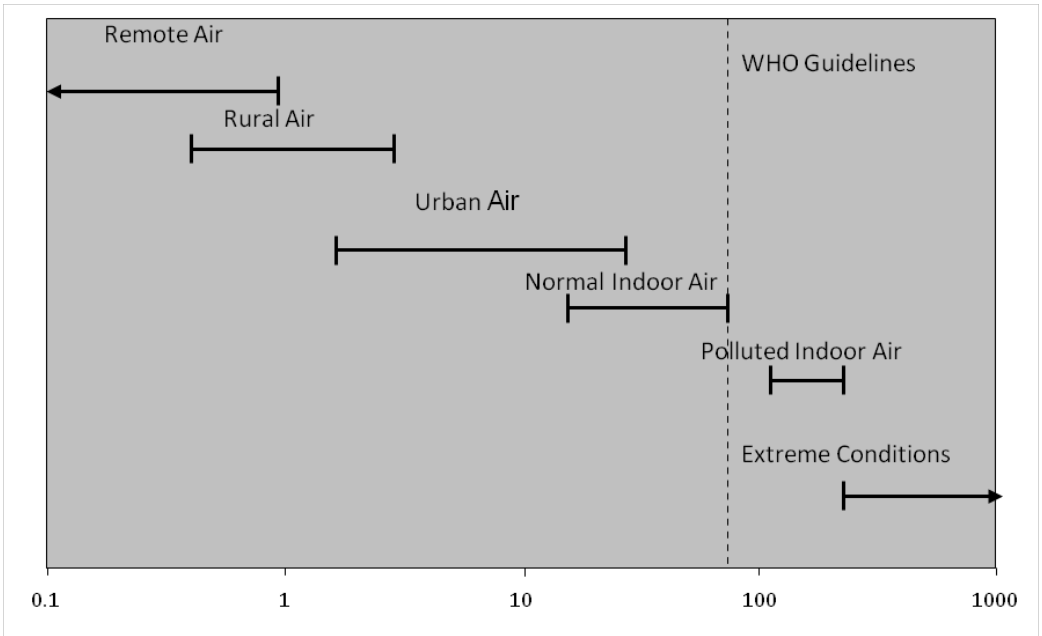

Figure A-3. Range of formaldehyde air concentrations (ppb) in different environments.

Notes: Graph is in logarithmic scale; "Normal indoor conditions," "polluted indoor conditions," and "extreme conditions" were not defined.
Source: Salthammer et al. (2010).

In addition, the Canadian indoor air data may overestimate formaldehyde levels in U.S. homes, because many residential homes in Canada use wood burning stoves more frequently and have tighter construction (due to colder winters), leading to less dilution of indoor emissions. The outdoor air levels, however, appear to have remained fairly constant over recent years, and the median outdoor level from the Canadian study (2.8 µg/m^3) (2.3 ppb) is very similar to the median of the U.S. monitoring data (2.83 µg/m^3) (2.3 ppb) in 1999.

Indoor air measurements combined with information about daily activity diaries have been used as surrogate of personal exposures. A recent study conducted with 41 children ages 9–12 years old in Australia concluded that although indoor air measurements from stationary monitors tended to slightly overestimate personal exposures, they were a good surrogate of personal exposures to children (Lazenby et al., 2012). The mean exposure from personal monitors ranged from <5 to 34 µg/m^3 (<4–26.3 ppb) with a mean of 13.7 µg/m^3 (11.1 ppb) (Lazenby et al., 2012).

Ingestion

Limited U.S. data indicate that concentrations in drinking water may range up to approximately 10 µg/L in the absence of specific contributions from the formation of formaldehyde by ozonation during water treatment or from leaching of formaldehyde from polyacetyl plumbing fixtures (WHO, 2002). In the absence of other data, one-half this concentration (5 µg/L) was judged

to be a reasonable estimate of the average formaldehyde in Canadian drinking water. Concentrations approaching 100 µg/L were observed in a U.S. study assessing the leaching of formaldehyde from domestic polyacetal plumbing fixtures, and this concentration was assumed to be representative of a reasonable worst case (WHO, 2002).

Formaldehyde has been used in the food industry for the preservation of dried foods, fish, certain oils and fats, and disinfection of containers (ATSDR, 1999). Formaldehyde is a natural component of a variety of foodstuffs (1995; IPCS, 1989). However, foods may be contaminated with formaldehyde as a result of fumigation (e.g., grain fumigation), cooking (as a combustion product), and release from formaldehyde resin-based tableware (IARC, 1995). Also, the compound has been used as a bacteriostatic agent in some foods, such as cheese (IARC, 1995). There have been no systematic investigations of levels of formaldehyde in a range of foodstuffs that could serve as a basis for estimation of population exposure (Health Canada, 2001). According to the limited available data, concentrations of formaldehyde in food are highly variable. In the few studies of the formaldehyde content of foods in Canada, the concentrations were within a range of <0.03–14 mg/kg (Health Canada, 2001). Data on formaldehyde levels in food have been presented by Feron et al. (1991) and WHO (1989) from a variety of studies, yielding the following ranges of measured values:

- Fruits and vegetables: 3–60 mg/kg
- Meat and fish: 6–20 mg/kg
- Shellfish: 1–100 mg/kg
- Milk and milk products: 1–3.3 mg/kg

Daily intake of formaldehyde was estimated by WHO (1989) to be in the range of 1.5–14 mg for an average adult. Similarly, Fishbein (1992) estimated that the intake of formaldehyde from food is 1–10 mg/day but discounted this on the belief that it is not available in free form. Although the bioavailability of formaldehyde from the ingestion of food is not known, it is not expected to be significant (ATSDR, 1999). Using U.S. Department of Agriculture (USDA) consumption rate data for various food groups, Owen et al. (1990) calculated that annual consumption of dietary formaldehyde results in an intake of about 4,000 mg or approximately 11 mg/day.

A.1.1.1. *Dermal Contact*

The general population may have dermal contact with formaldehyde-containing materials, such as some building products and cosmetics (see Section 1.2 for the details on these products). Generally, though, dermal contact is more of a concern in occupations that involve handling concentrated forms of formaldehyde, such as those occurring in embalming and chemical production.

A.2. TOXICOKINETICS OF INHALED AND ENDOGENOUS FORMALDEHYDE

This chapter presents specific information on the toxicokinetics [absorption, distribution, metabolism, and excretion (ADME)] of inhaled and endogenously produced formaldehyde from human and experimental animal studies. Although toxicokinetics is typically discussed in a sequential manner [i.e., with absorption defined as delivery to the blood; distribution describing delivery to the target tissue(s); metabolism outlining conversion to a more-or-less active chemical species, often metabolism occurs in liver, target tissue elsewhere; and excretion documenting tissue clearance and removal processes], the primary site of action of inhaled formaldehyde is at the portal of entry (POE), specifically within the upper respiratory tract (URT). Therefore, this section will first discuss the uptake (also referred to as "absorption" in the formaldehyde literature) of inhaled formaldehyde into the URT tissue, and its transport, metabolism, and removal within the POE. Following this is a description of what is known regarding the absorption of formaldehyde from the POE into the blood and the potential for distribution of exogenous formaldehyde to systemic sites, along with a discussion of formaldehyde metabolism and excretion processes that may occur outside of the POE.

Formaldehyde is produced endogenously during normal cellular metabolism and as a byproduct of lipid peroxidation, or as a product in the catabolism of other chemicals introduced through dietary, environmental, or pharmaceutical sources. Therefore, discussions of inhaled formaldehyde require a consideration of the potential impact of endogenous formaldehyde on its toxicokinetics, as well as on its toxicity. The available evidence on the metabolism and kinetics of endogenous formaldehyde is discussed within each of the following subsections specifically as it pertains to the toxicokinetics of exogenous formaldehyde.

In the last subsections, the available toxicokinetic models of formaldehyde are presented.

A.2.1. Toxicokinetics of Inhaled Formaldehyde at the Portal of Entry (POE)

Formaldehyde is a highly reactive, highly water soluble, respiratory irritant, towards which the human body has developed several detoxification and removal processes at the site(s) of first contact (e.g., nasal passages for inhalation). Thus, this discussion of the toxicokinetics of inhaled formaldehyde at the POE is organized according to the most likely sites of first contact between inhaled formaldehyde and biological materials, in the context of the known anatomy and potential elimination processes of the respiratory tract tissues. Several of the key considerations for evaluating the toxicokinetics of inhaled formaldehyde at the POE in the rat nose are represented schematically in Figure A-4. The respiratory tract is divided broadly as (1) upper respiratory tract (URT), which includes the nasal cavity, pharynx, and larynx and (2) the lower respiratory tract (LRT) comprising the trachea, bronchi, and lungs. Species differences in the structure of the airways, as well as the composition of the surface epithelium at various nasal locations, are important considerations to keep in mind when interpreting results in rodents and extrapolating observations to humans. Nasal passages, starting from anterior to posterior, are lined by four

different types of epithelia: (1) squamous or keratinized, stratified (nasal vestibule); (2) transitional or nonciliated cuboidal/columnar; (3) respiratory or ciliated pseudostratified cuboidal/columnar (main chamber and nasopharynx); and (4) olfactory (dorsal and dorsoposterior nasal cavity) (Harkema et al., 2006). It is important to note that rodents and humans differ in the distribution of nasal epithelial surfaces. For example, the olfactory epithelium in rats and mice makes up approximately 50-52% and 45-47%, respectively, of the nasal cavity surface area, whereas in humans, it makes up only 3% (Sorokin, 1988; Gross et al., 1982).

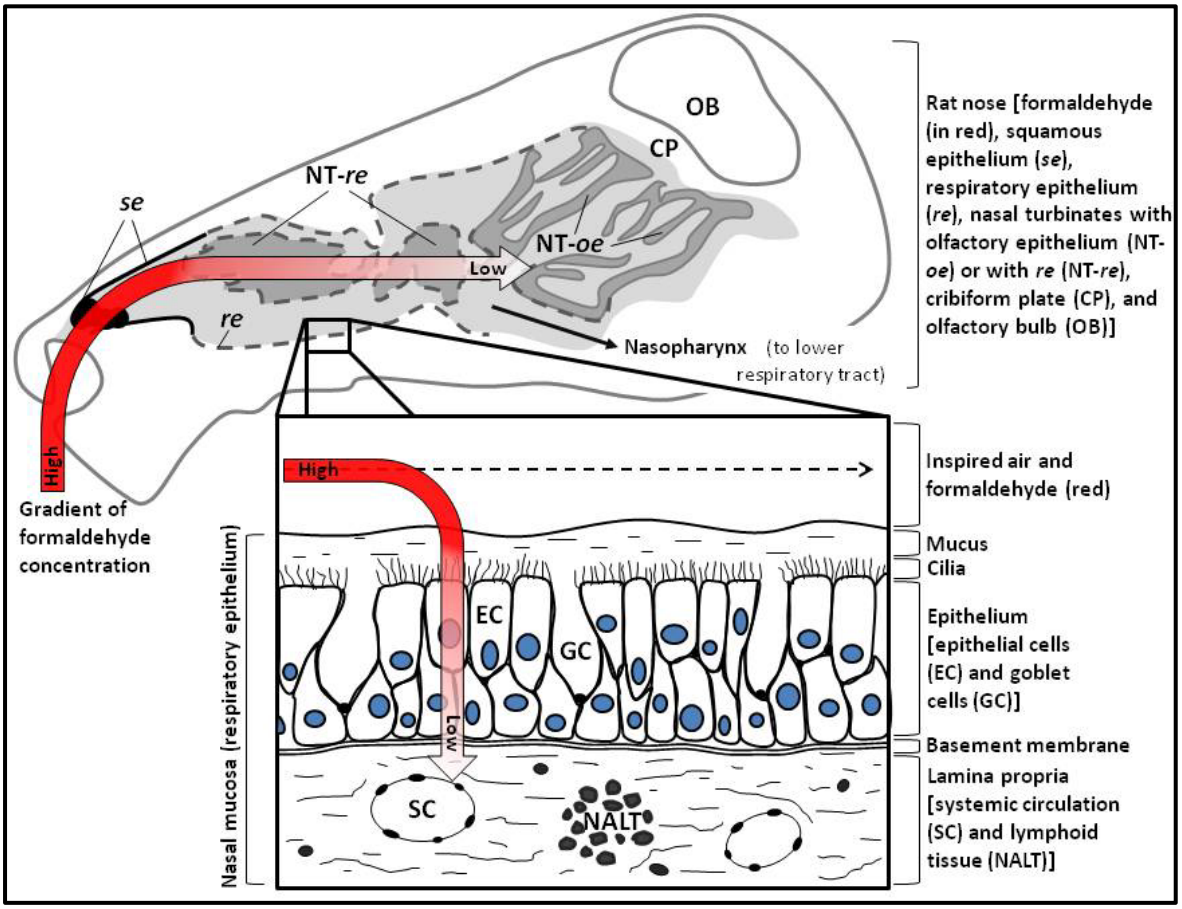

Figure A-4. Schematic of the rat upper respiratory tract depicting the gradient of formaldehyde concentration formed following inhalation exposure, both from anterior to posterior locations, as well as across the tissue depth.
Modeling based on observations in rodents predicts a similar pattern of distribution in humans. Drawn based in part on images by NRC (**2011**) and Harkema et al. (**2006**). Note: other components (e.g., naris; transitional epithelium) have been omitted to increase clarity.

A.2.2. Spatial Distribution of Tissue Uptake of Formaldehyde at the Portal of Entry

The distribution of inhaled formaldehyde within the URT and LRT can provide information useful to interpreting any potential toxicity. The nasal passages in humans are generally similar to

other mammalian species. One key difference, however, is that humans and nonhuman primates have nasal passages adapted for both oral and nasal (oronasal) breathing, as opposed to obligate nasal breathing in rodents. A second key difference regards the shape and complexity of the nasal turbinates, with relatively simple shapes in humans, and complex, folded patterns in rodents. In general, these differences provide better protection of the rodent LRT against inhaled toxicants than is provided to the human LRT (Harkema et al., 2006).

Indirect measurement studies

Much of what is known regarding the uptake of formaldehyde is based on indirect measurements of formaldehyde-induced changes and/ or molecular interactions, or removal of formaldehyde from the air. This is because, in biological systems, formaldehyde exists as total or analyzable formaldehyde, which includes free and reversibly bound (acid-labile) forms (Heck et al., 1982). Conventional methods cannot directly measure low levels of free formaldehyde with certainty in tissues and body fluids. Additionally, carbonyl impurities such as acetone, formaldehyde and acetaldehyde are present even in quartz distilled water and may interfere in the measurements (Esterbauer et al., 1982). Uptake of formaldehyde (defined as retention within the respiratory tract tissue), based on rough estimates determined from the amount of formaldehyde removed from the air, indicate that majority large percentage of formaldehyde is removed from inhaled air by the URT.

Indirect estimates of formaldehyde uptake, based on interactions with cellular materials, have been made in experimental animals, including monkeys (Casanova et al., 1991; Monticello et al., 1989), dogs (Egle, 1972), and rats (Kimbell et al., 2001b; Chang et al., 1983; Heck et al., 1983; Kerns et al., 1983) as shown in Table A-5.

Table A-5. Dosimetry and response of formaldehyde in experimental animals by indirect measurements

Reference and species	Exposure and analysis	Observations	
Casanova et al. (1991); Monkeys, rhesus; male, n=9; 8.74 kg; 4.6 yr old	0.86, 2.46, 7.38 mg/m^3 for 6-hr [^{14}C]CH_2O from [^{14}C]PFA. Estimated the amount of DNA-protein crosslinks (DPX) formed in various tissues	***DPX Levels***	***Area of the respiratory tract***
		Highest	Middle turbinate mucosa
		Lower	Anterior lateral wall/septum and nasopharynx
		Very low	Larynx/trachea/carina
		None	Maxillary sinuses and lungs
Monticello et al. (1989) Monkeys, rhesus; male, *n*=9; 4-6 yrs; 6-7 kg	7.4 mg/m^3, 6 hrs/d; 5 d/wk; 1 or 6 wk CH_2O from PFA. Animals injected with [^{3}H]-Thd, sacrificed, histoauto-radiography of cell proliferation measured	***Proliferation***	***Area of the respiratory tract***
		Significant	Nasal passages
		Minimal	Lower respiratory tract

Reference and species	Exposure and analysis	Observations				
		None	Maxillary sinuses			
Egle (1972) Dogs/Mongrel; Male and female; *n*=4; 13-19 kg	150 to 350 mg/m^3 CH_2O vapors from formalin; nose-only inhalation from a respirometer; animals preanesthetized; aldehydes analyzed by a colorimetric method	***Uptake at all ventilation rates and concentrations***				
		Total respiratory tract (TRT)	≈100%			
		URT- inhalation	100%			
		URT- inhalation + exhalation	≈100%			
Heck et al. (1983); Rats, Fischer; Male, *n*=3; 18--250 g	Radioactivity immediately after 6hr exposure to [^{14}C]CH_2O from [^{14}C]PFA, each averaging 3 exposures and 4 rats at 6.2, 12.3, 18.5, or 29.5 mg/m^3		**Equivalents of [^{14}C] in various tissues (µmol/g)[a] or mg/m^3**			
			6.15	**12.3**	**18.5**	**29.5**
		Nasal Mucosa	0.59 ± 0.18	1.15 ± 0.29	1.78 ± 0.4	2.28 ± 0.61
		Trachea	0.26 ± 0.13	0.39 ± 0.13	0.36 ± 0.09	0.40 ± 0.13
		Plasma	0.05 ± 0.01	0.08 ± 0.01	0.10 ± 0.04	0.11 ± 0.05

[a]Values, representing mean ± SD, were extracted from graphical data using GrabIT software.
CH2O, formaldehyde; PFA, paraformaldehyde; DPX, DNA-protein crosslinks.

As shown in Table A-5, Casanova et al. (1991) used DNA-protein crosslinks (DPX) levels as a measure of regional dosimetry of formaldehyde in monkeys exposed to formaldehyde by inhalation assuming that the rate of crosslink formation depends on the concentration of formaldehyde delivered at the portal of entry tissues. They subjected rhesus monkeys to a single 6-hr exposure of formaldehyde over a range (0.9–7.4 mg/m^3) and concluded based on the observed pattern of DPX formation that formaldehyde uptake primarily occurs in nasal passages involving middle turbinates, to a smaller extent in the nasopharynx and trachea, but not in maxillary sinuses or lungs (Casanova et al., 1991). Monticello et al. (1989) predicted the uptake of formaldehyde based on other indirect measures such as cell proliferation in monkeys repeatedly exposed to 7.4 mg/m^3 formaldehyde, 6 hrs/day, 5 days/wk for 1 or 6 wks. They concluded that formaldehyde uptake primarily occurs in nasal passages and middle turbinates, to a smaller extent in the nasopharynx and trachea, with evidence of increased proliferation in proximal regions of the bronchi, but no indication of effects in the maxillary sinuses. In dogs exposed to formalin vapors, almost 100% of inhaled formaldehyde is retained in the URT, indicating that little, if any, inhaled formaldehyde would reach the LRT, and this is independent of respiration rate, tidal volume, and inhaled formaldehyde concentration (Egle, 1972).

Similarly, radiolabeling studies, exemplified by Heck et al. (1983) in rats show that the majority of the labeled formaldehyde is retained within the nasal passages and, to a far lesser extent, within the other parts of the URT and proximal LRT, with no evidence of significant distribution into plasma. However, because formaldehyde is incorporated into the one-carbon (1C)

pool (see discussion later in this section), possibly facilitating its distribution in a toxicologically-inactive form, neither the distribution of radiolabel nor the estimated retention are interpreted to provide a clear picture of the spatial distribution of inhaled formaldehyde within the respiratory tract tissues. Notably, long-term exposure of rats to formaldehyde for 30 months induced lesions in the nasal cavity and proximal trachea (Kerns et al., 1983). Kimbell et al. (2001b) predicted the uptake of formaldehyde in the nasal passages of F344 rats, rhesus monkeys and humans to be respectively, 90%, 67% and 76% using the computational fluid dynamics (CFD) modeling. Similar to these predictions for rats, Morgan et al. (1986c) demonstrated that rat nasal passages scrubbed nearly all of the inhaled formaldehyde (on average ≈97%). In rats, the evidence suggests that higher concentrations of formaldehyde are taken up in the respiratory mucosa as compared to the olfactory mucosa (Casanova-Schmitz et al., 1984b; Swenberg et al., 1983a).

Extrapolation using fluid dynamic modeling

There are no studies available in the literature that directly addressed uptake of formaldehyde into the respiratory tract of humans. However, a few modeling studies based on findings in rodents report estimated uptake of inhaled formaldehyde in humans (Kimbell et al., 2001b; Kimbell and Subramaniam, 2001; Overton et al., 2001). Kimbell et al. (2001b), using a three-dimensional, CFD model of the nose, predicted human nasal uptake of approximately 76% of the inhaled formaldehyde at unidirectional steady-state nasal inspiratory flow corresponding to sleeping activity, decreasing to 58% under heavy exercise activity. Overton et al. (2001) modeled overall uptake in the entire respiratory tract and predicted that 95% of inhaled formaldehyde is retained in the respiratory tract in general in any activity state. A detailed description of modeling efforts in humans and monkeys (and rats) is provided in Appendix B.2.2. Overall, dosimetric modeling studies in humans have shown close agreement with observations of exposed rodents: namely, that 90–95% of inhaled formaldehyde is retained in the URT (Kimbell et al., 2001b; Overton et al., 2001; Subramaniam et al., 1998).

Relationship of formaldehyde uptake to endogenous levels and prior exposure

Heck et al (1982) developed a gas chromatography-mass spectrometry (GC-MS) method to measure total or analyzable formaldehyde, which includes both free as well as reversibly bound formaldehyde [hydrated formaldehyde bound to glutathione (GSH) and tetrahydrofolate (THF)]. However, this method does not measure irreversibly bound formaldehyde. Based on this method, endogenous formaldehyde levels were 1.5–4.3 folds higher at the POE (i.e., nasal mucosa; ≈12.6 µg/g or 0.42 mM) than in other tissues (i.e., testes<liver<brain) (Heck et al., 1982). It remains to be determined how this may affect the local toxicokinetics of inhaled formaldehyde.

Heck et al. (1983) also examined the effect of prior exposure to formaldehyde on tissue levels of formaldehyde in rats. As shown in Table A-6, no statistically significant changes in total formaldehyde levels in the nasal mucosa were observed following 10-day exposure of F344 rats to 7.4 mg/m^3 formaldehyde (Heck et al., 1982), suggesting that formaldehyde exposure does not

distinguishably augment total levels of formaldehyde in POE tissues. However, rats and mice appear to differ in the uptake of formaldehyde following repeated inhalation exposure to formaldehyde. Prior, short-term exposure to high levels of formaldehyde in rats did not alter uptake of formaldehyde into the respiratory mucosa during a subsequent exposure. This was based on comparisons between a single exposure to 18.5 mg/m^3 in naïve rats compared to repeated exposures in rats exposed to the same dose of formaldehyde for the previous 9 days (Heck et al., 1983). In a different study, Chang et al. (1983) also observed similar uptake in preexposed as well as naïve rats; however, mice responded differently, with naïve mice exhibiting more radioactivity uptake than preexposed mice (see Table A-6). The authors concluded that since mice tend to lower their minute volume with repeated exposures to formaldehyde, they tend to have less absorption, hence less radioactivity compared to naïve mice. So comparing the results in rats, which do not alter their minute volume as mice do, it was suggested that repeated exposure does not affect the uptake of formaldehyde in nasal cavity of rats (Chang et al., 1983).

Table A-6. Comparison of formaldehyde uptake at the portal of entry with single or repeated inhalation exposure

Reference and design	Exposure and analysis	Observations
Heck et al. (1982) Rats, Fischer Male, *n*=8 200–250 g	7.4 mg/m^3 [^{13}C] CH_2O (from PFA) for 6 hrs/d; 10-d exposure; chamber inhalation; CH_2O measured as PFPH derivative by GC/MS	Nasal mucosa levels total[a] CH_2O (μg/g[b]) *Unexposed* 12.6 ± 2.7 — *Exposed* 11.7 ± 3.6
Heck et al. (1983) Rats, Fischer Male, n=3; 180–250 g	Two groups: (a) *preexposure*; (b) *naïve*; On Days 1-9: group a) received 18.5 mg/m^3 CH_2O (from PFA); whole body exposure, 6 hrs/d; group b): no preexposure. On Day 10: groups a and b received [^{14}C] CH_2O (from PFA) for 6 hrs, nose-only exposure. Tissue homogenates counted with LSC for $^{14}CO_2$ trapped in ethanolamine in 2-methoxy-ethanol counted for radioactivity.	Equivalents of ^{14}C in respiratory mucosa (μg /g[c]) naïve rats 67.5 ± 9.2 preexposed 64.4 ± 7.6 (No significant difference)
Chang et al. (1983) Rats, Fischer; Male, N=3; 180-200 g Mice, B6C3F1 Male, N=3; 26 g	i) preexposure: 7.4 or 18.4 mg/m^3 unlabeled CH_2O from PFA, 6 hrs/d, 4-days whole-body exposure; on 5th day $^{14}CH_2O$ from PFA, 6 hrs ii) naïve animals: $^{14}CH_2O$, 6 hrs from PFA	Radioactivity in nasal cavity: preexposed rats = naïve rats Radioactivity in nasal cavity: naïve mice > pretreated mice

[a]Total formaldehyde includes free plus reversibly bound formaldehyde.

[b]Data from Heck et al. (1982) given in μmols/g is converted to μg/g by the equation: μmols × 30 = μg/g (30 is the molecular weight of formaldehyde).

[c]Data from Heck et al. (1983) given in nmols/g is converted to converted to μg/g by the equation: (nmol/g /1,000) × 30 = μg/g) (30 is the molecular weight of formaldehyde).

CH_2O, formaldehyde; PFA, paraformaldehyde; PFPH, pentafluorophenylhydrazine; GC/MS, gas chromatography/mass spectrometry; LSC, liquid scintillation counting; CO_2, carbon dioxide.

Summary of spatial distribution of POE uptake

To summarize, a majority of inhaled formaldehyde is rapidly absorbed and retained in the URT based on CFD modeling studies in humans (Kimbell et al., 2001b; Kimbell and Subramaniam, 2001; Overton et al., 2001; Subramaniam et al., 1998), indirect or direct measurements in monkeys (Monticello et al., 1989; Casanova et al., 1988), and direct measurements in dogs (Egle, 1972) and rats (Kimbell et al., 2001b; Chang et al., 1983; Heck et al., 1983; Kerns et al., 1983), despite the anatomical and physiological differences between species, such as obligate nose breathing in rodents (rats and mice) and oronasal breathing in primates (monkeys and humans) (Harkema et al., 2006; Schreider, 1986). As demonstrated in monkeys and rats, and as modeled in humans, a concentration gradient of inhaled formaldehyde follows an anterior to posterior distribution, with high concentrations of formaldehyde distributed to squamous, transitional and respiratory epithelia, and less uptake by olfactory epithelium, and very little or no formaldehyde reaching more distal sites such as the larynx or lung. Further, at inhaled concentrations as high as 7.4 mg/m^3, exogenous exposure does not appreciably change the levels of formaldehyde over the endogenous levels in the nasal mucosa (Heck et al., 1982). Also, repeated exposures to formaldehyde do not alter the tissue formaldehyde levels in rats, but naïve mice do show higher tissue uptake than preexposed mice, which is attributed to species differences in minute volume and response to irritant gases (Chang et al., 1983).

A.2.3. Tissue Penetration of Formaldehyde Within the Upper Respiratory Tract

Within the URT, penetration of formaldehyde follows initial interaction with the mucociliary apparatus followed by diffusion into the epithelial cell layer where it can be metabolized. Important details to consider in evaluating formaldehyde nasal dosimetry and toxicity are the differences in the types of epithelium lining the nasal surfaces. As described earlier, there are striking differences in the amount of olfactory epithelium and respiratory epithelium present between the noses of rats, which have a highly complex sense of smell, compared to humans, who use the nose primarily used for breathing. In all species, air (and formaldehyde) must first pass over squamous, transitional, and respiratory epithelium before coming in contact with olfactory epithelium. This section will focus on the interaction and fate of inhaled formaldehyde in the URT.

Formaldehyde interaction with the mucociliary layer

The mucociliary apparatus of the URT is the first line of defense against airborne agents in that it may entrap, neutralize, and remove particulates and airborne chemicals from inspired air (Morgan et al., 1983). The mucociliary apparatus is comprised of three layers: a thick mucus layer (epiphase) at the top, a watery fluid layer (hypophase) in the middle, and a ciliated epithelial layer at the bottom (Schlosser, 1999). Inhaled formaldehyde must pass through the mucus layer covering the URT before it can react with the cellular components in this region.

The respiratory mucus is composed of 97% water, 2–3% glycoproteins, 0.3–0.5% fats, and about 0.1–0.5% soluble proteins (Bogdanffy et al., 1987). Formaldehyde gas (unhydrated) is highly soluble in water, in which it hydrolyzes to a reversible hydrated form called methanediol or methylene glycol with a half-life of 70 milliseconds and with an equilibrium constant $[CH_2O]/[CH_2(OH)_2]$ of 4.5×10^{-4} at 22°C (Sutton and Downes, 1972). In aqueous solution, most of the formaldehyde (99.9%) exists as methanediol in an equilibrium with free (0.1%) formaldehyde (Fox et al., 1985). Thus, formaldehyde is first hydrated in nasal mucus to form methanediol, which subsequently interacts with the nasal mucociliary apparatus (Priha et al., 1996; Bogdanffy et al., 1986). Physical-organic chemistry studies of the reaction of formaldehyde with amines (and presumably other biological nucleophiles) have conclusively demonstrated that the unhydrated or free form of formaldehyde, but not the hydrated form or methanediol is the reactive species (Abrams and Kallen, 1976). Methanediol is either transported to the underlying tissue (presumably by diffusion) or it is removed within nasal mucus by convective flow and subsequent ingestion. Schlosser (1999) estimated that 22–42% of the absorbed formaldehyde in rodents is removed by mucus flow.

Airborne pollutants and reactive gases have been shown to decrease mucus flow rates in several animal models (as reviewed in as reviewed in Wolff, 1986). Degradation in the continuity or function of this mucociliary apparatus can impair clearance of inhaled pollutants at the portal of entry. For example, Morgan et al. (1983) have shown that a single exposure of 18.45 mg/m^3 formaldehyde in Fischer rats causes mucostasis (cessation or severe slowing of mucus flow) in several regions of the nasoturbinates. Repeated exposure (6 hours/day for 1–9 days) results in ciliastasis (loss of ciliary activity) occurring with greater frequency and across more regions of the nasoturbinates in subsequent days of exposure. Thus, continued exposure would be expected to result in an increased uptake, as well as an altered deposition of inhaled formaldehyde within the URT tissue. Further, Morgan et al. (1986c) also reported that rats exposed 6 hours daily for 3 weeks showed increase in mucostasis extending from anterior to posterior regions at the 18.45 mg/m^3 dose; however, at lower doses (0.6–7.4 mg/m^3) the effect was either undetectable or less severe. In addition, Morgan et al. (1986c) showed an increase in mucus flow at lower concentrations after 4 days exposure, but not after 6 days to 0.6 mg/m^3 formaldehyde. Thus, there are some uncertainties regarding the occurrence of mucostasis at lower concentrations of formaldehyde exposure.

In addition, as methanediol and free formaldehyde are transported through the mucociliary apparatus, the free formaldehyde is known to bind to soluble proteins such as albumin in the nasal mucus (Bogdanffy et al., 1987). Similarly, the nasal lining fluid contains antioxidants, including the thiol GSH with which formaldehyde is known to interact, likely eliciting a transient GSH depletion during and following formaldehyde exposure. However, it is unclear to what extent inhaled formaldehyde interacts with soluble and insoluble factors within the mucociliary layer and whether reactive byproducts may be formed by these interactions. Importantly, endogenous formaldehyde

produced during normal cellular metabolism is unlikely to be present at appreciable levels in the mucus, and thus, would not be expected to participate in similar reactions. Interactions with soluble proteins are expected to further reduce the amount of formaldehyde available to react with cellular materials. As such, alterations in the levels of soluble proteins within the mucus could substantially affect tissue uptake.

Formaldehyde diffusion into the epithelial cell layer

The less reactive methanediol is better able to penetrate tissues, while the free formaldehyde reacts with the macromolecules. However, when the free formaldehyde (≈0.1%) is used up, a fraction of methanediol (from the 99.9%) will convert to free formaldehyde so that the equilibrium of methanediol with free formaldehyde (i.e., 99.9:0.1 ratio) is maintained in the aqueous media (Fox et al., 1985). However, several uncertainties exist regarding the transition of inhaled formaldehyde from the mucociliary layer to the underlying epithelium. Although direct experimental evidence is lacking, the biochemical properties of formaldehyde make it likely that inhaled formaldehyde (in the hydrated or anhydrated form) undergoes passive transport, via simple diffusion, across biological membranes. Thus, higher extracellular formaldehyde levels would be expected to result in increased diffusion into the cell owing to the concentration gradient formed. However, this concentration gradient may be affected by endogenous formaldehyde levels because in humans, as in other animals, formaldehyde is an essential metabolic intermediate in all cells (Thompson et al., 2009).

Enzymatic metabolism of formaldehyde within cells of the URT

Formaldehyde, either from exogenous sources (inhaled air) or endogenous sources (enzymatic and nonenzymatic mechanisms as well as that released endogenously from metabolism of xenobiotics), can be metabolized by several different enzyme pathways. Based on studies of endogenous formaldehyde and in vitro enzyme inhibition experiments (Teng et al., 2001), and as summarized in Figure A-5, formaldehyde has been shown to be predominantly metabolized to formate by GSH-dependent class III alcohol dehydrogenase (ADH3; also described as formaldehyde dehydrogenase or FDH) and by a minor pathway involving mitochondrial aldehyde dehydrogenase 2 (ALDH2) which is GSH-independent. Catalase may also be involved, to a minor extent, in oxidizing formaldehyde, especially under conditions when hydrogen peroxide is formed (Uotila and Koivusalo, 1974).

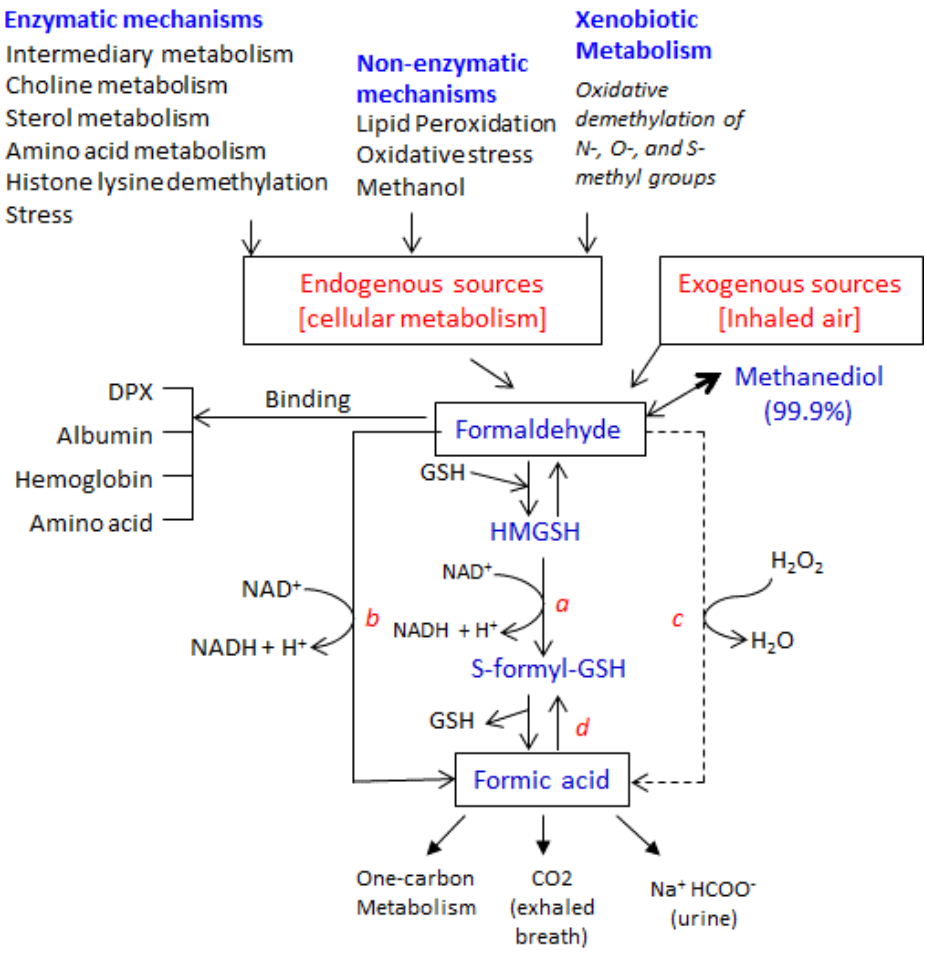

Figure A-5. Metabolism of formaldehyde.

Abbreviations: CO2, carbon dioxide; DPX, DNA-protein crosslinks; GSH, glutathione; H_2O, water; H_2O_2, hydrogen peroxide; HMGSH, hydroxymethylglutathione; NAD^+, nicotinamide adenine dinucleotide (oxidized); NADH, nicotinamide adenine dinucleotide (reduced); Na^+HCOO^-, sodium formate. Enzymes: a, alcohol dehydrogenase-3 (ADH3); b, aldehyde dehydrogenase 2 (ALDH2); c, catalase; d, S-formyl-GSH hydrolase.
Adapted from NTP (2010).

Both ADH3 and ALDH2 enzymes have been found across different species and in a broad range of tissues, including the nasal mucosa (Reviewed in Reviewed in Thompson et al., 2009). In rodents, both ADH3 and ALDH2 exhibit region-specific differences in the nose, in that the specific activity of ADH3 is twice higher in the olfactory mucosa than in respiratory mucosa, while the specific activity of ALDH2 is 5–8 times higher in respiratory than in olfactory tissue (Bogdanffy et al., 1986; Casanova-Schmitz et al., 1984a). In rats, higher levels of ADH3 activity have been reported in the cytoplasm of the respiratory and olfactory epithelial cells and in the nuclei of olfactory sensory cells, as compared to other regions of the nasal mucosa (Keller et al., 1990). These enzymes are enriched in the nasal tissues presumably to protect the underlying tissues against respired toxicants. This highlights a significant barrier to the penetration of inhaled

formaldehyde beyond the respiratory epithelium and a means by which these same cells can rapidly metabolize formaldehyde produced endogenously within the cell (Uotila and Koivusalo, 1974).

The ADH3-mediated pathway of formaldehyde oxidation involves a two-step enzymatic reaction but is preceded by the rapid and reversible nonenzymatic binding of formaldehyde to GSH, which results in the formation of S-hydroxymethylglutathione (HMGSH) or the glutathione hemiacetal adduct. In the first of a two-step enzymatic reaction, ADH3 converts HMGSH to S-formylglutathione (S-formyl-GSH) in the presence of the co-factor, nicotinamide adenine dinucleotide (NAD^+). In the second step, another enzyme S-formyl-GSH-hydrolase converts S-formyl-GSH to formate with the concomitant release of free GSH. Under physiological conditions, cellular NAD^+ levels are two orders of magnitude higher than NADH (reduced form of NAD^+) and intracellular GSH levels are high enough (in millimolar concentrations) to favor rapid oxidation of HMGSH to formate (Svensson et al., 1999; Meister and Anderson, 1983). Because of this rapid metabolism, formaldehyde is likely to have a short half-life in biological systems. As previously mentioned, and given the importance of this major detoxification pathway, individual variations in GSH levels within the nasal mucosa are of particular importance in formaldehyde metabolism.

ADH3 shows comparable kinetics across rats and humans. As shown in Table A-7, the affinity (K_m) of purified human liver ADH3 for HMGSH is 6.5 μM (Uotila and Koivusalo, 1974) and 4.5 mM for rat liver (Casanova-Schmitz and Heck, 1983). Hedberg et al. (2000) demonstrated that the kinetics of ADH3 in human buccal tissue lysates are in close agreement with those reported for purified human liver ADH3 (Uotila and Koivusalo, 1974). This is comparable to the rat respiratory and olfactory mucosal K_m values in the presence of GSH as well as the K_m of ADH3 from rat liver soluble fraction (2.6 μM) (Casanova-Schmitz et al., 1984a). In contrast, the affinity of ALDH2, presumably represented in the absence of GSH is several-fold lower than ADH3 (Siew et al., 1976). Thus, at lower concentrations of formaldehyde ADH3 is the dominant formaldehyde detoxification pathway. The K_m of ADH3 is in close agreement across species and tissue types, including the nasal mucosa, all of which exhibit similar responses to GSH depletion (i.e., in the absence of GSH, ALDH family members oxidize formaldehyde, which is associated with mitochondrial ALDH2). Both ADH3- and ALDH2-mediated pathways oxidize formaldehyde to formic acid (formate). ADH3 is also known to catalyze the NADP-dependent reduction of the endogenous nitrosylating agent S-nitrosoglutathione (GSNO) and is also referred to as S-nitrosoglutathione reductase (GSNOR) (Jensen et al., 1998).

Table A-7. ADH3 kinetics in human and rat tissue samples and cultured cells

Source	Km (μM)	Vmax (nmol/mg protein x min)	References
Purified human liver ADH3	6.5	2.77 ± 0.12	Uotila and Koivusalo (1974)
Rat respiratory mucosal homogenate (+GSH)	2.6 ± 2.6	0.90 ± 0.24	

Source	Km (μM)	Vmax (nmol/mg protein x min)	References
Rat respiratory mucosal homogenate (– GSH)	481 ± 88	4.07 ± 0.35	Casanova-Schmitz et al. (1984a)
Rat olfactory mucosal homogenate (+GSH)	2.6 ± 0.5	1.77 ± 0.12	
Rat olfactory mucosal homogenate (– GSH)	647 ± 43	4.39 ± 0.14	
Rat liver (+ GSH)[a]	4.5 ± 1.9[a]	2.0 ± 0.3	
Human buccal tissue (+ GSH)	11 ± 2	2.9 ± 0.6	Hedberg et al. (2000)
Human buccal tissue (– GSH)	360 ± 90	1.2 ± 0.7	

[a]Soluble fraction of rat liver homogenate.

Formate can undergo three possible outcomes: (1) enter the one-carbon pool for use in the synthesis of DNA and proteins (aka "metabolic incorporation"), (2) become further oxidized to CO_2 and eliminated in exhaled air, or (3) be excreted in urine (Figure A-5).

One-carbon metabolism

As summarized in Figure A-6, the tetrahydrofolate (THF)-mediated eukaryotic one-carbon (1C) metabolism involves an inter-connected network which is highly compartmentalized between the cytosol, mitochondria, and nucleus (Reviewed in Reviewed in Tibbetts and Appling, 2010). A majority of the 1C metabolism takes place in the mitochondria followed by the cytosol and nucleus. In the cytoplasmic 1C metabolism, de novo synthesis of purines and thymidylate, and remethylation of homocysteine to methionine takes place. The 1C metabolism in the mitochondrial compartment involves formylation of methionyl-tRNA, oxidation of one-carbon donors, such as serine, glycine, sarcosine, and dimethylglycine (DMG). In addition, mitochondria contribute 1C units for cytoplasmic 1C metabolism in the form of formate. The mitochondrial and cytoplasmic pathways are connected by serine, glycine and formate which are the 1C donors. The nuclear compartment of 1C metabolism predominantly provides de novo synthesis of dTMP from dUMP.

Some of the steps in the cytosolic and mitochondrial 1C metabolism are common. Formate, formed from the metabolism of formaldehyde, enters the 1C pool and is either oxidized to CO_2 and eliminated in exhaled breath or is used in protein and DNA synthesis. As shown in Figure A-6, formate is combined with THF whereby its 1C group is transferred to THF forming 10-formyl-THF (10-CHO-THF), mediated by the enzyme 10-HCO-THF-synthetase. The 10-CHO-THF is then oxidized by CHO-THF dehydrogenase to CO_2 and H_2O and eliminated in the exhaled breath, with the release of THF which can be reused for binding with formic acid. Alternatively, 10-CHO-THF can also be converted through two-steps of reversible reactions to 5,10-methenyl-THF (CH^+-THF) to 5,10-methylene-THF (CH_2-THF). Serine, derived from glycolytic intermediates, is the main source of 1C units. Serine combined with THF is converted reversibly by the enzyme serine hydroxymethyl transferase (SHMT) to glycine and CH_2-THF. Further, the enzyme methylene tetrahydrofolate reductase (MTHFR) converts CH_2-THF to 5-methyl-THF (CH_3-THF). The 1C metabolism products -CH_2-THF and CH_3-THF utilize their one-carbon units, respectively, in DNA (dTMP) and protein (methionine) biosynthetic pathways (metabolic incorporation).

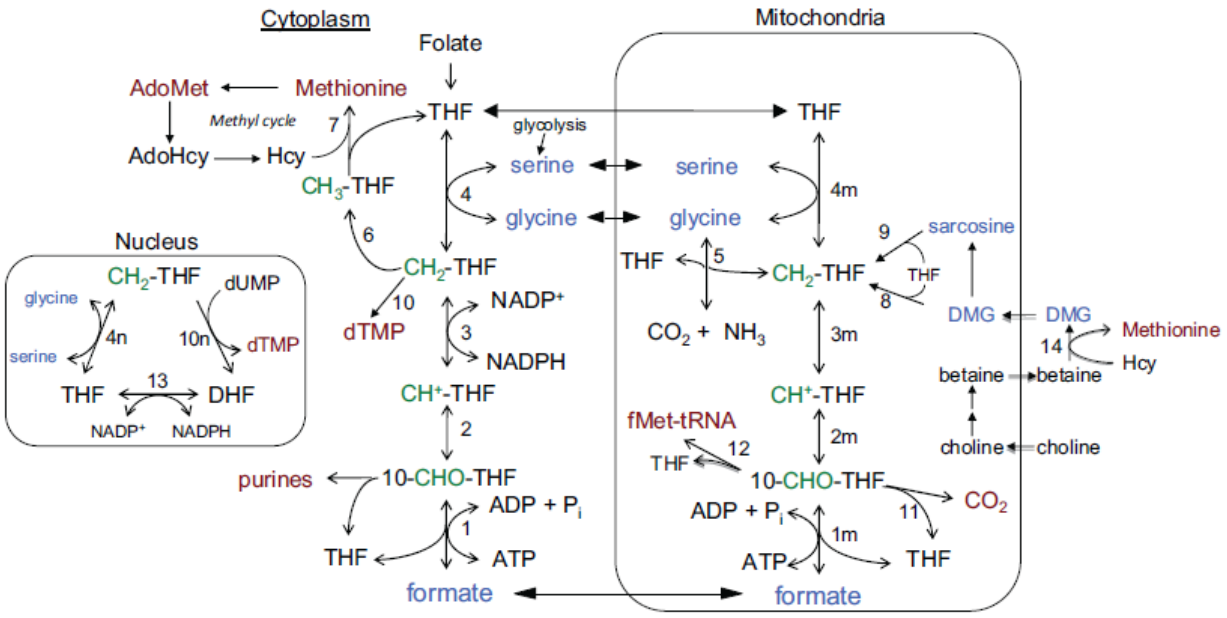

Figure A-6. Compartmentalization of mammalian one-carbon metabolism. The end products, donors, and activated units carried by tetrahydrofolate (THF) of the 1C metabolism are shown in red, blue, and green, respectively. Note that reactions 1–4 are common in both the cytoplasmic and mitochondrial (m) compartments, while reactions 4 and 10 are present in the nucleus (n). Enzymes catalyzing the reactions: 1: 10-formyl-THF synthetase; 2: 5,10-methenyl-THF (CH+-THF) cyclohydrolase; 3: 5,10-methylene-THF (CH_2-THF) dehydrogenase; 4, 4n, and 4m: serine hydroxymethyltransferase (SHMT); 5: glycine cleavage system; 6: 5,10-methylene-THF reductase; 7: methionine synthase; 8: dimethylglycine dehydrogenase (DMGDH); 9: sarcosine dehydrogenase (SDH); 10 and 10n: thymidylate synthase; 11: 10-formyl-THF dehydrogenase (only the mitochondrial activity of this enzyme is shown, but it has been reported in both compartments in mammals); 12: methionyl-tRNA formyltransferase; 13: dihydrofolate (DHF) reductase; 14: betaine-homocysteine methyltransferase. Abbreviations: AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine; Hcy, homocysteine.

Source: Tibbetts and Appling (2010).

The rate of formate metabolism depends on the availability of dietary folic acid, which is the main source of THF. It is also important to note that levels of folate intermediates and folate-dependent enzymes show some differences in rats and primates (see Table A-8).

Table A-8. Levels of folate intermediates, activity of folate-dependent enzymes, and the rate of oxidation of formate in the liver of various species

Folate intermediate/folate-dependent enzyme	Rat	Monkey	Human
10-formyl-THF (nmoles/g of liver)	4.6 ± 1.3	10.5 ± 0.8	3.3 ± 0.5
Tetrahydrofolate (nmoles/g of liver)	11.4 ± 0.8	7.4 ± 0.8	6.5 ± 0.3

Folate intermediate/folate-dependent enzyme	Rat	Monkey	Human
5-CH_3-THF (nmoles/g of liver)	9.3 ± 0.6	7.6 ± 1.1	6.0 ± 0.7
10-formyl-THF synthetase (nmoles of product/min/mg protein)	65.9 ± 0.0	142 ± 16	75.0 ± 8.7
10-formyl-THF dehydrogenase (nmoles of product/min/mg protein)	88.3 ± 1.7	33.0 ± 4.0	23.0 ± 2.2
5,10-CH_2-THF reductase (nmoles of product/min/mg protein)	1.21 ± 0.07	0.22 ± 0.02	0.42 ± 0.07
Serine hydroxymethyl transferase (nmoles of product/min/mg protein)	10.8 ± 0.6	17.1 ± 9.7	18.5 ± 0.7
Dihydrofolate reductase (nmoles of product/min/mg protein)	19.8 ± 1.3	4.1 ± 0.7	0.74 ± 0.17
Methionine synthase (nmoles of product/min/mg protein)	0.09 ± 0.007	0.09 ± 0.012	0.10 ± 0.008
Rate of formate oxidation (mg/kg/hr)	78	40	0

Source: Skrzydlewska (2003)

As shown in Table A-8, the normal hepatic THF levels of monkeys and humans are 1.5 and 1.75-fold lower than the levels in rats. Also, the levels of 10-formyl-THF-dehydrogenase levels are 2.67- and 3.83-fold lower in monkeys and humans, respectively, compared to the levels in rat liver, which might cause an accumulation of formate in primates since there is decreased oxidation of formate to CO_2. Thus, primates oxidize formate less efficiently than rats (Skrzydlewska, 2003).

Interaction of formaldehyde with cellular macromolecules in the URT

As mentioned earlier, it has been shown that "free" formaldehyde (i.e., the 0.1% of total formaldehyde that does not exist in the form of methanediol) reacts with macromolecules (Abrams and Kallen, 1976). However, it is unclear whether methanediol in certain hydrophobic matrices (e.g., crossing biological membranes, etc.) could be converted to a more reactive form and available to interact with cellular materials. Inhaled formaldehyde interacts at the portal of entry with the nasal passages, and these interactions can be either noncovalent (reversible) or covalent (irreversible).

Noncovalent interactions:

Formaldehyde is reversibly bound to GSH and THF in the cells forming the glutathione hemithioacetal adduct or hydroxymethylglutathione (HMGSH) adduct and 5, 10-CH_2-THF adducts. Levels of the cellular antioxidant glutathione are abundant in the cell ≈5 mM with which formaldehyde readily forms the hemiacetal adduct. The dissociation constant for the hemiacetal and CH_2-THF adducts are approximately 1.5 mM (Uotila and Koivusalo, 1974) and ≈30 μM, respectively (Kallen and Jencks, 1966a, b). Based on in vitro experiments formaldehyde has been shown to reversibly bind to human and rat nasal mucus, in particular the fraction containing albumin (Bogdanffy et al., 1987).

Covalent binding

Formaldehyde covalently binds to protein, DNA, DNA and proteins forming protein adducts, DNA adducts, DNA-protein crosslinks (DPX), and DNA-DNA crosslinks (DDX). A complication that

has been explored in some of these studies is that inhaled formaldehyde can also be metabolized and incorporated into DNA and proteins via the 1C pool.

Protein adducts

Formaldehyde has been shown to bind to histones and chromatin forming N^6-formyllysine (Edrissi et al., 2013a) and a major source of this adduct has been shown to result from endogenous formaldehyde. Further, in rats exposed to various inhalation concentrations of ^{13}C-labeled formaldehyde (0.9–11.2 mg/m^3), a concentration-dependent increase in ^{13}C-labeled N^6-formyllysine, which was distinguished from endogenous N^6-formyllysine, was detectable in the total proteins as well as in protein fractions from different cellular compartments (cytoplasmic, membrane, and nuclear) of the respiratory epithelium (Edrissi et al., 2013a).

DNA-protein Crosslinks

Formaldehyde-induced DNA-protein crosslinking occurs predominantly between the epsilon-amino groups of lysine, especially the N-terminus of histones, and exocyclic amino groups of DNA (Lu et al., 2008). Several analytical methods including radiolabeled formaldehyde have been used to evaluate DPX formation in experimental animals. Earlier experiments have shown that inhalation of F344 rats to 2.46–36.93 mg/m^3 of ^{14}C-formaldehyde (6 hours/day, 2 days) caused a significant increase in the radioactivity of interfacial (IF) DNA[1], representing DPX, observed in tissue homogenates from respiratory but not olfactory epithelium at ≥ 7.38 mg/m^3 (Casanova-Schmitz and Heck, 1983). Formaldehyde-induced DPX levels have been shown to have concentration-dependence in both monkeys (0.86 to 7.37 mg/m^3) (Casanova et al., 1991) and rats (0.37–12.1 mg/m^3) (Casanova et al., 1994; Casanova et al., 1989). In both rodents and monkeys there was a nonlinear concentration-response for DPX formation, which has been attributed to saturation of detoxification enzymes at high concentrations (Casanova et al., 1991; Casanova et al., 1989). In monkeys, the DPX distribution pattern in the nasal passages following formaldehyde inhalation was in the order of middle turbinates > anterior lateral wall/septum > maxillary sinuses and lungs (Casanova et al., 1991), which corresponded to the location and proliferative response. In rats the DPX distribution pattern was in the order of lateral meatus > medial and posterior meatus (Casanova et al., 1994), which corresponded to the high and low tumor incidence sites in the respiratory tract (Monticello et al., 1989). This is possibly due to the differences in the anatomy of nasal passages and breathing patterns of these two species.

Recently, Lai et al. (2016) developed a method that distinguishes deoxyguanosine-methyl-cysteine (dG-Me-Cys), a DPX formed from exogenous formaldehyde from that formed from endogenous formaldehyde (see Table A-9). In monkeys exposed to 7.4 mg/m^3 of ^{13}C-labeled

[1] During a typical DNA extraction of tissue homogenates, the DNA separated into aqueous phase is termed aqueous (AQ) DNA, while the DNA trapped in the protein precipitate from the interphase (between aqueous and organic phases) was washed, treated with protein kinase and reextracted to get the interfacial DNA (IF DNA).

formaldehyde for 2 days, both exogenous and endogenous DPXs were detectable, with the levels of exogenous DPXs being 2.8-fold less than the endogenous DPX adducts. In contrast, only endogenous DPXs were detectable in air-exposed monkeys. In rats, a higher dose of 18.5 mg/m^3 formaldehyde exposed for 1, 2, or 4 days was tested. DPX levels in nasal tissues were detected and were comparable for endogenous and exogenous formaldehyde among rats exposed 1 or 2 days, but at 4 days, DPX levels from exogenous formaldehyde had increased 5-fold above those from endogenous formaldehyde. Similarly, DPX levels from exogenous formaldehyde increased between 7 days and 28 days in rats exposed to 2.5 mg/m^3.

Using in vitro studies, Yu et al. (2015b) have shown that DPX such as, dG-CH_2-cysteine or dG-CH_2-GSH can undergo hydrolytic degradation to give rise to hm-dG monoadducts under physiological pH and temperature conditions. These results provide a mechanism which explains why formaldehyde-induced DPX are removed within 12.5−24 hrs in cultured human epithelial cell lines (Quievryn and Zhitkovich, 2000) and lymphoblasts (Craft et al., 1987). However, the in vivo studies by Lai (2016) did not replicate this phenomenon. These more precise studies have shown that in rats exposed to 2.5 mg/m^3 labeled formaldehyde for 28 days, at 1-week postexposure, 87% of the exogenous DPX were retained in the nasal tissues, suggesting a slow repair of these bulky adducts. The potential implications of this for dose-response modeling are discussed in Appendix B.2.2.

Table A-9. Summary of endogenous and exogenous DNA-protein crosslinks in nasal tissues of rats following inhalation exposure of $^{13}CD_2$-labeled formaldehyde

Reference and design	Exposure and analysis	Exposure duration	CH2O conc.	Observations	
Lai et al. (2016); Monkeys, cynomolgus; *N*=4-6.	0 (air control) or 7.4 mg/m^3 [$^{13}CD_2$]-CH_2O from PFA by inhalation; 6 hrs/d; for 2 d; whole-body exposure; nasal tissue collected; DNA extracted with DNAzol reagent, dG-Me-Cys purified on HPLC and analyzed by nano-LC/ESI/MS-MS.		(mg/m^3)	Endogenous adducts	Exogenous adducts
				dG-Me-Cys/10^8 dG	
		2 d	0	3.59 ± 1.01	ND
		2 d	7.4	3.76 ± 1.50	1.36 ± 0.20
Lai et al. (2016); Rats, F344; *N*=4-6.	0 (air control) or 18.5 mg/m^3 [$^{13}CD_2$]-CH_2O from PFA by inhalation; 6 hrs/d; for 1,2, or 4 d; whole-body exposure; nasal tissue collected; DNA extracted with DNAzol reagent, dG-Me-Cys purified on HPLC and analyzed by nano-LC/ESI/MS-MS.	Exposure Duration	(mg/m^3)	Endogenous adducts	Exogenous adducts
				dG-Me-Cys/10^8 dG	
		4 d	0	6.50 ± 0.30	ND
		1 d	18.5	4.42 ± 1.10	5.52 ± 0.80
		2 d	18.5	4.28 ± 2.34	4.69 ± 1.76
		4 d	18.5	3.67 ± 0.80	18.18 ± 7.23
Lai et al. (2016); Rats, F344; *N*=4-6.	Rats, inhalation exposure to 2.5 mg/m^3 CH_2O for 7 or 28 d and allowed to recover for 1 or 7 d PE. Nasal tissue collected and DNA extracted at the given time points and analyzed for dG-Me-Cys adducts as above.	Exposure Duration	(mg/m^3)	Endogenous adducts	Exogenous adducts
				dG-Me-Cys/10^8 dG	
		7 d	2.5	4.78 ± 0.64	0.96 ± 0.17
		28 d	2.5	4.51 ± 1.48	2.46 ± 0.44

Reference and design	Exposure and analysis	Exposure duration	CH2O conc.	Observations	
		28 d + 1 d PE	2.5	3.78 ± 0.69	2.12 ± 1.00
		28 d + 7 d PE	2.5	3.51 ± 0.16	2.14 ± 1.02

Abbreviations: PFA, paraformaldehyde; LC, liquid chromatography; MS, mass spectrometry; HPLC, high performance liquid chromatography; CH2O, formaldehyde; DPX, DNA-protein crosslinks; dG-Me-Cys, deoxyguanosine-methyl-cysteine; PBMC, peripheral blood mononuclear cell; ESI, electron spray ionization; PE, post-exposure.

Distinguishing covalent binding of formaldehyde from metabolic incorporation

Few studies from the same research group addressed the issues of differentiating covalently bound (i.e., DPX formation) versus metabolically incorporated formaldehyde in rats exposed to formaldehyde by inhalation (Casanova and Heck, 1987; Casanova-Schmitz et al., 1984b; Casanova-Schmitz and Heck, 1983).

Casanova-Schmitz et al. (1984b) used dual isotope labeling as a way to partially distinguish between covalent binding (DPX formation) and metabolic incorporation of formaldehyde. In this approach, male F344 rats were exposed to a mixture of ^{3}H- and ^{14}C-labeled formaldehyde for 6 hours at exposure concentrations ranging from 0.37−18.42 mg/m^3, a day after exposure to nonradioactive formaldehyde with the same dose range. The IF DNA was extracted from respiratory and olfactory mucosa, and the $^{3}H/^{14}C$ ratios of different phases of DNA extraction (i.e., AQ DNA and IF DNA) were measured. It is important to note that formaldehyde loses the hydrogen atom during oxidation reactions (i.e., metabolic incorporation), but not during covalent binding to DNA. Therefore, the $^{3}H/^{14}C$ ratio in a sample that contains adducts and crosslinks should be higher than in a sample that primarily contains DNA with metabolically incorporated formaldehyde.

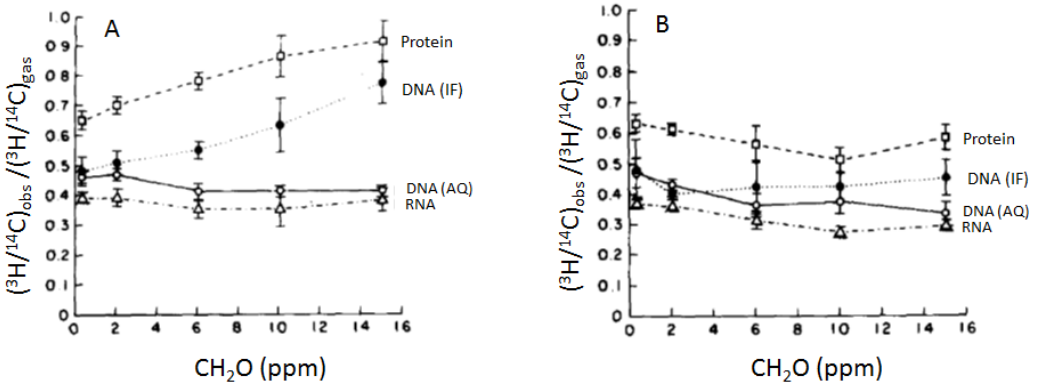

Figure A-7. Metabolic incorporation and covalent binding of formaldehyde in rat respiratory tract. 3H/14C ratios in macromolecular extracts from rat respiratory mucosa (A) and olfactory mucosa (B) following 6-hour exposure to ^{14}C- and 3H-labeled formaldehyde (0.3, 2, 6, 10, and 15 ppm, corresponding to 0.37, 2.46, 7.38, 12.3, 18.42 mg/m^3, respectively).

Source: Adapted from Casanova-Schmitz et al. (1984)

As seen in panel A of Figure A-7, Casanova-Schmitz et al. (1984) report that IF DNA from nasal respiratory mucosa has a significantly higher $^{3}H/^{14}C$ ratio (*Y*-axis) than the aqueous phase (AQ) DNA, with a nonlinear dose response of IF DNA at exposure concentrations equal to or greater than 2.46 mg/m^3. These data suggest that IF DNA has significantly more ^{3}H, a phenomenon likely explained by additional ^{3}H-formaldehyde molecules present as DPXs prior to DNA extraction. These crosslinks were due to exogenous formaldehyde that could be attributed to DPX. The $^{3}H/^{14}C$ ratio was linearly increased for the organic fraction, suggesting covalent binding of formaldehyde to respiratory mucosa proteins. In contrast, olfactory mucosa did not show increased $^{3}H/^{14}C$ ratio in the IF DNA or AQ DNA or proteins phase as a function of formaldehyde concentration (panel B, Figure A-7). In total, these data suggest that the radiolabeling observed following formaldehyde exposure in rats results from both covalent binding and metabolic incorporation in the nasal mucosa, but not the olfactory mucosa (Casanovaschmitz et al., 1984). The respiratory mucosa from unexposed rats appears to contain 15% of DNA as IF DNA (Casanova-Schmitz and Heck, 1983), possibly as endogenous DPX.

DNA monoadducts

Another form of formaldehyde-induced covalent DNA modifications is hydoxymethyl-DNA (hm-DNA) adducts or DNA monoadducts. Five studies conducted in one laboratory used $^{13}CD_2$-formaldehyde in experimental rats and monkeys coupled with an LC/MS approach to distinguish hm-DNA adducts formed by endogenous and exogenous formaldehyde (Yu et al., 2015b; Lu et al., 2011; Moeller et al., 2011; Lu et al., 2010a), as summarized in Table A-10. In this method, hm-DNA adducts formed by exogenous $^{13}CD_2$-formaldehyde are distinguished from unlabelled endogenous hm-DNA adducts based on the differences in their typical *m*/*z* ratio (Lu et al., 2012b). As shown in Table A-10, both exogenous and endogenous N^2-hydroxymethyl-deoxyguanosine (N^2-hm-dG) adducts were detected in nasal tissues of cynomologous monkeys exposed to 2.34 or 7.5 mg/m^3 $^{13}CD_2$-formaldehyde for 2 days, and across several rat studies testing exposures ranging from 0.9–18.7 mg/m^3 formaldehyde for several hours up to 28 days (Yu et al., 2015a; Yu et al., 2015b; Lu et al., 2011; Lu et al., 2010a). Notably, however, these studies demonstrate that the levels of endogenous N^2-hm-dG adducts were several folds higher than corresponding exogenous adducts in nasal tissue.

While these studies provide the first insights into the relationship between endogenous and exogenous DNA monoadducts, further study may help to clarify some remaining uncertainties. For example, the potential involvement of different types of DNA monoadducts, as well as their specific toxicodynamic roles (e.g., for cancer development), remain poorly understood. Of the studies which used inhalation exposure to ^{13}C-labeled formaldehyde, only Lu et al. (2010a) quantified other adduct types; interestingly, while the authors detected $^{13}CD_2$-labeled N^2-hm-dG adducts and dG-CH_2-dG crosslinks, they did not detect N^6-hydroxymethyl-deoxyadenosine (N^6-hm-dA) adducts in the nasal epithelium of rats exposed for 1 or 5 days (12.3 mg/m^3) to exogenous formaldehyde.

However, the same group reported the formation of both N^2-hm-dG (most of the tissues) and N^6-hm-dA monoadducts (only in bone marrow) in rats that were dosed by gavage with ^{13}C-labeled methanol, which is a precursor of formaldehyde (Lu et al., 2012b). Similarly, a different research group reported that rats dosed subcutaneously with nitrosamines (Wang et al., 2007b), which are precursors to formaldehyde, and smokers (Wang et al., 2009a) both exhibit N^6-hm-dA monoadducts in peripheral tissues. Thus, additional sensitive evaluations of dA monoadducts, particularly following longer term formaldehyde exposure and preferably in humans, may be informative. Also of interest, it is important to keep in mind that the experiments conducted to date involve comparisons of endogenous adduct levels, which would represent steady-state formaldehyde levels after having built up over time from the continuous presence of endogenous formaldehyde, to exogenous adduct levels resulting from short-term and/ or episodic (e.g., 6 hr/day) exposures. As an illustration, with exogenous exposure for 6-hr/day, multiple weeks or longer could be needed to reach steady-state levels, and, even so, those levels could be roughly expected to be four-fold lower than if a continuous (24 hrs/d) exogenous exposure occurred at the same concentration. The recent study by Yu et al. (2015b) begins to address this, noting that "quasi-steady-state" levels appear to be nearing after 6hr-day exposure to 2.46 mg/m^3 formaldehyde for 28 days; however, exogenous adducts were still substantially increased with 28 days, as compared to 21 days of exposure, and exogenous adducts reached ≈37% of endogenous adducts (1.05 versus 2.82 adducts/10^7 dG, in contrast to the ≈14% observed after 7 days of exposure) under this scenario. Considering these data at 2.46 mg/m^3, the comparability of endogenous versus exogenous adducts relevant to lifetime exposure scenarios would be informed by additional studies incorporating a range of experiments and formaldehyde concentrations that span short, episodic exposures to more constant, long-term exposures.

Table A-10. Summary of endogenous and exogenous DNA monoadducts in nasal tissue of monkeys and rats following inhalation exposure of $^{13}CD_2$-labeled formaldehyde

Reference and design	Exposure and analysis[a]	Portal of entry tissues	CH_2O exposure conc. (mg/m^3)	Observations	
				Endogenous adducts	Exogenous adducts
Moeller et al (2011); Monkeys, cynomolgus; *n*=3	2.34 or 7.5 mg/m^3 [$^{13}CD_2$]-CH_2O; 6 hrs/d; for 2 d (whole-body exposure); sacrificed immediately after exposure; tissues collected.	Nasal maxilloturbinates		N^2-hm-dG/10^7dG	
			2.34	2.50 ± 0.40	0.26 ± 0.04
			7.5	2.05 ± 0.54	0.41 ± 0.05

Reference and design	Exposure and analysis[a]	Portal of entry tissues	CH_2O exposure conc. (mg/m^3)	Observations	
				Endogenous adducts	Exogenous adducts
Yu et al. (2015b); Monkeys, cynomolgus; *n*=4	0 (air control), 2.4 or 7.5 mg/m^3 $[^{13}CD_2]$-CH_2O generated from $[^{13}CD_2]$PFA; nose-only exposure; 6 hrs/d for 2 consecutive days; Sacrificed immediately after exposure; maxilloturbinates (Animal #1) and all other nasal tissues (Animal #2) were collected.	Nasal maxilloturbinates	2.4	2.50 ± 0.44	0.26 ± 0.04
			7.5	2.05 ± 0.54	0.41 ± 0.05
		Nasal dorsal mucosa	0	3.81 ± 1.19	ND
			7.5	3.62 ± 1.28	0.40 ± 0.07
		Nasal nasopharynx	0	3.48 ± 0.53	ND
			7.5	3.62 ± 1.34	0.33 ± 0.10
		Nasal septum	0	3.75 ± 0.32	ND
			7.5	3.56 ± 0.69	0.39 ± 0.15
		Nasal anterior maxillary	0	4.21 ± 0.53	ND
			7.5	3.80 ± 0.91	0.34 ± 0.12
		Nasal posterior maxillary	0	3.95 ± 0.74	ND
			7.5	3.46 ± 1.05	0.36 ± 0.16
		Trachea carina	0	2.69 ± 0.95	ND
			7.5	2.33 ± 1.12	ND
		Trachea proximal	0	2.35 ± 1.05	ND
			7.5	2.35 ± 1.05	ND
Lu et al. (2010a); Rats, Fisher; Male, *n*=5–8	12.28 mg/m^3 $[^{13}CD_2]$-CH_2O generated from $[^{13}CD_2]$PFA; 6 hrs/day, 1 or 5 days; nose-only exposure; Sacrificed immediately after exposure; tissues collected.	Nasal tissue[b,c]	Exposure duration	Endogenous adducts	Exogenous adducts
				N^2-hm-dG/10^7 dG	
			1-d	2.63 ± 0.73	1.28 ± 0.49
			5-d	2.84 ± 1.13	2.43 ± 0.78
				N^6-hm-dA/10^7 dA	
			1-d	3.95 ± 0.26	ND
			5-d	3.61 ± 0.95	ND
				dG-CH_2-dG/10^7 dG	
			1-d	0.17 ± 0.05	0.14 ± 0.06
			5-d	0.18 ± 0.06	0.26 ± 0.07
Lu et al. (2011); Rats, Fischer; *n*=5–6	$[^{13}CD_2]$-CH_2O from $[^{13}CD_2]$PFA; 6 hrs, nose-only exposure; Sacrificed immediately after exposure; tissue collected.	Nasal tissue	Exposure concentration (mg/m^3)	Endogenous adducts	Exogenous adducts
				N^2-hm-dG adducts/10^7 dG	
			0.9 ± 0.25	3.62 ± 1.33	0.039 ± 0.019
			2.5 ± 0.12	6.09 ± 3.03	0.19 ± 0.08
			7.1 ± 0.62	5.51 ± 1.06	1.04 ± 0.24
			11.2 ± 2.71	3.41 ± 0.46	2.03 ± 0.43
			18.7 ± 2.58	4.24 ± 0.92	11.15 ± 3.01

Reference and design	Exposure and analysis[a]	Portal of entry tissues	CH_2O exposure conc. (mg/m^3)	Observations	
				Endogenous adducts	Exogenous adducts
Yu et al. (2015b); Rats, Fischer, male; *n*=8–9	0 (air control) or 2.46 mg/m^3 [$^{13}CD_2$]-CH_2O from [$^{13}CD_2$]PFA; nose-only exposure; 6 hrs/d for 7, 14, 21, or 28 consecutive days; postexposure recovery for 6, 24, 72, and 168 hrs. Sacrificed immediately after exposure at indicated time points; tissues collected.	Nasal epithelium	**Exposure duration**	Endogenous adducts	Exogenous adducts
				N^2-hm-dG/10^7 dG	
			Air control	2.84 ± 0.54	ND
			7 d	2.51 ± 0.63	0.35 ± 0.17
			14 d	3.09 ± 0.98	0.84 ± 0.17
			21 d	3.34 ± 1.06	0.95 ± 0.11
			28 d	2.82 ± 0.76	1.05 ± 0.16
			6 hrs PE	2.80 ± 0.58	0.83 ± 0.33
			24 hrs PE	2.98 ± 0.70	0.80 ± 0.46
			72 hrs PE	2.99 ± 0.63	0.63 ± 0.12
			168 hrs PE	2.78 ± 0.48	0.67 ± 0.20

[a]Tissue DNA was extracted, reduced with sodium cyanogen borohydride ($NaCNBH_3$), digested and analyzed by nano-UPLC-MS/MS.
[b]Nasal respiratory epithelium from the right and left sides of the nose and the septum.
[c]Exogenous N^6-hmdA adducts were not detected in any tissues; exogenous N^2-hm-dG and dG-dG crosslinks were detected only in nasal tissues.
Abbreviations: CH2O, formaldehyde; D_2, deuterium; MS, mass spectrometry; PE, postexposure; PFA, paraformaldehyde; ND, not detected; N^2-hm-dG, N^2-hydroxymethyl-deoxyguanine; N^6-hm-dA, N^6-hydroxymethyl-deoxyadenine; dG-CH_2-dG, dG-dG crosslinks; UPLC, ultra-pressure liquid chromatography.

Unknown contribution of potential interactions with other nasal mucosa elements

Formaldehyde is likely to interact with other components of the nasal mucosa depending on the concentration and duration of exposure. A small amount of inhaled formaldehyde, converted predominantly to methanediol, is expected to penetrate the epithelial cell layer and react with the basement membrane or with constituents of the *lamina propria*, including components of the connective tissue/extracellular space, mucus gland components, lymphoid components, and vascular components. Andersen et al. (2008) examined the gene expression in different tissue compartments of male F344 rats exposed to formaldehyde concentrations ranging from 0.9–18.5 mg/m^3 by inhalation exposure. They reported that at low concentrations (0.9–2.5 mg/m^3) formaldehyde is likely to react with the extracellular components of the cells at or near the cell membrane, while at higher doses (7.5–18.5 mg/m^3) responses are observed in both extracellular and intracellular sites involving more genes in the response. The gene expression data from this study suggests the possibility for a potential interaction of formaldehyde with other nasal mucosa components.

Removal of inhaled formaldehyde from the POE

The main processes for removing inhaled formaldehyde from the URT involve clearance in the mucus and metabolism to formic acid. Formic acid can enter the 1C pool and may either be oxidized to CO_2 or incorporated metabolically into nucleic acids and proteins carrying the 1C units

through THF derivatives. Formate can also be absorbed into circulation, reach the kidneys, and be excreted in urine.

Summary of penetration, metabolism and removal of inhaled formaldehyde within the URT tissue

In summary, as inhaled formaldehyde enters the URT it interacts with the mucociliary apparatus which is the first line of defense. In nasal mucus, most of the formaldehyde is rapidly converted to methanediol (≈99.9%) and a minor fraction remains as free formaldehyde (≈0.1%). Inhaled formaldehyde induces mucostasis and ciliastasis in rat nasal mucociliary apparatus extending from the anterior to posterior regions of nasal cavity depending on the concentration and duration of exposure (Morgan et al., 1986a). However, as previously noted, uncertainties remain regarding the pattern of induced mucostasis, or the complete lack thereof, at low levels of formaldehyde exposure. Methanediol is assumed to be better able to penetrate the tissues, while free formaldehyde reacts with the macromolecules. It is assumed that the equilibrium is rapid, hence that the methanediol:free formaldehyde equilibrium ratio is maintained (Fox et al., 1985). However, uncertainties remain regarding the net impact of the transition of inhaled formaldehyde from the mucociliary layer to the underlying epithelium due to the presence of endogenous formaldehyde, which is a component of normal cellular metabolism. In the URT, formaldehyde is predominantly metabolized by glutathione-dependent class III alcohol dehydrogenase (ADH3) and by a minor pathway involving aldehyde dehydrogenase 2 (ALDH2) to formate. Formate can either enter the one-carbon pool leading to protein and nucleic acid synthesis, or is further metabolized to CO_2 and eliminated in expired air or excreted in urine unchanged.

Formaldehyde can interact with macromolecules either by noncovalently binding to GSH, THF, or albumin in nasal mucus or covalently forming DPX, DDX, hm-DNA adducts, or protein adducts. In rats and monkeys, formaldehyde exposure results in a concentration-dependent increase in DPX. Metabolic incorporation studies with ^{14}C-formaldehyde have shown both covalent binding and metabolic incorporation in nasal tissues (Casanova and Heck, 1987; Casanova-Schmitz et al., 1984b). Distribution patterns in the nasal passages correspond to the tumor incidence locations in rats and to proliferative response patterns in both rats and monkeys. Hence, DPX has been used as a surrogate biomarker of exposure for risk assessment. Inhaled formaldehyde induces a concentration-dependent increase in N^2-hm-dG adducts in the nasal passages of monkeys and rats. Recently, analytical methods have been developed that can distinguish N^2-hm-dG adducts formed from exogenous sources from those formed from endogenous sources. Notably, endogenous N^2-hm-dG adduct levels are much higher than exogenous monoadduct levels in animals, because formaldehyde is known to be produced continuously during normal cellular metabolism. It has been suggested that N^2-hm-dG adducts could be used as a marker of exposure in risk assessment. However, this use might be compromised by several methodological issues in the adduct isolation and analysis.

A.2.4. Modifying Factors and Specific Uncertainties Regarding the Toxicokinetics of Inhaled Formaldehyde Within the POE

Many factors could influence the uptake and removal of inhaled formaldehyde at the POE. Distribution and tissue penetration of inhaled formaldehyde could both be significantly modified as a result of changes in environmental factors or tissue alterations induced by prolonged exposure. Similarly, metabolic detoxification of formaldehyde and clearance from the URT are dependent upon a number of cofactors and proteins that may be modified by changes to the environment or by prolonged exposure. Finally, modeling indicates that endogenous formaldehyde has the potential to impact on the toxicokinetics of inhaled formaldehyde. This section will not include a description of every potential modifying factor, but will attempt to highlight those interpreted to be most important or controversial, particularly those that may be essential to interpreting differences between experimental animals and humans.

Adjustments to account for reflex bradypnea in rodent studies

Reflex bradypnea (RB) is a protective reflex that allows rodents—but not humans—to significantly reduce their inhalation exposures to URT irritants such as formaldehyde. When an irritating concentration of formaldehyde triggers RB via the trigeminal nerve, rodents have an immediate decrease in respiratory rate and minute volume, and thus a marked decrease in formaldehyde exposure. Their RB persists until the exposure ends although the strength of the response in the initial minutes after exposure begins can be much stronger than later in the exposure. Kane and Alerie (1977) showed a maximal response in naïve mice of 13.7% decreased respiration rate from exposure to 0.55 ppm formaldehyde. This increased slightly to 15.6% in mice preexposed for 3 days. Consequently, a rodent study may not be health protective for humans unless the chamber concentrations or minute volume are adjusted to account for the rodents' reduced formaldehyde exposure. However, existing models and dose-response analyses have not accounted for this effect.

Unfortunately, it is not known if or when rodents develop a tolerance to formaldehyde and resume normal breathing. Considering that Chang and Barrow (1984) reported that F-344 rats experienced RB throughout 10 days of formaldehyde exposure, it may be appropriate to adjust short-term rodent exposure concentrations to make them health protective for humans. Because a long-term RB study has never been performed for formaldehyde or any other URT irritant, there is no way of knowing whether similar adjustment is warranted for subchronic and/or chronic rodent studies. This is a significant data gap.

Modification due to effects of exposure on nasal mucosa function

Several events reported to occur after inhalation exposure to formaldehyde have the potential to modify the toxicokinetics of formaldehyde in the URT during subsequent exposure scenarios. Important among these factors are dynamic tissue modeling, changes in mucociliary

clearance, reduction in minute volume, and changes in glutathione levels and glutathione-mediated ADH3 activity.

Functional changes in the respiratory epithelium could have significant effects on the subsequent uptake of inhaled formaldehyde. Squamous metaplasia, a tissue conversion that is an adaptive response that occurs in nasal epithelium exposed to toxic levels of formaldehyde, has been observed in rats exposed to ≥2.46 mg/m^3 formaldehyde for longer than 18 months. This type of dynamic tissue remodeling of nasal airways can affect formaldehyde dosimetry, as squamous metaplastic tissue is known to absorb considerably less formaldehyde than other epithelial types (Kamata et al., 1997). This is of critical concern for dosimetric modeling efforts, which typically rely on results from simulations of acute, rather than prolonged, exposure. The highest flux levels of formaldehyde in simulations of the rat nose in Kimbell et al. (2001b) are estimated in the region just posterior to the nasal vestibule. A consequence of squamous metaplasia is to "push" the higher levels of formaldehyde flux toward the more distal regions of the nose (Kimbell et al., 1997b). Uncertainties in the modeling of formaldehyde dosimetry are presented by Subramaniam et al. (2008) and are discussed in the PBPK Section (see Appendix B.2.2). A similar concern is raised regarding the observation that exposure affects the integrity and/or function of the mucociliary layer, as previously discussed (see Section A.2.3).

Exposure-induced changes to factors involved in the detoxification of formaldehyde could also affect its toxicokinetics during a subsequent challenge. The enzyme ADH3 is central to the metabolism of formaldehyde; however, exposure to formaldehyde in turn alters the activity of ADH3-dependent critical metabolic pathways. For example, transcription of ADH3 correlates with the proliferative states in human oral keratinocytes (Nilsson et al., 2004; Hedberg et al., 2000). In rodent lung, an increase in ADH3 activity affects other ADH3 substrates involved in protein modification and cell signaling (Que et al., 2005). Other pathways of ADH3 include oxidation of retinol and long-chain primary alcohols and reduction of S-nitrosoglutathione (GSNO). GSNO can accelerate ADH3-mediated formaldehyde oxidation and, likewise, formaldehyde increases ADH3-mediated GSNO reduction nearly 25-fold. Because GSNO is an endogenous bronchodilator and reservoir of nitric oxide (NO) activity, ADH3-mediated reduction of GSNO can cause a deregulation of NO (Reviewed in Reviewed in Thompson et al., 2010).

Similarly, glutathione is essential to detoxification of formaldehyde through the major pathway. GSH is present in most cells at levels far in excess of formaldehyde. In humans, the HMGSH levels are high since circulating GSH concentrations are ≈50 times higher than formaldehyde (Sanghani et al., 2000). It is estimated that ≈50–80% of formaldehyde in animal cells is reversibly bound to GSH (Uotila and Koivusalo, 1989) and to a minor extent bound reversibly to tetrahydrofolate (Heck et al., 1982). Inhaled formaldehyde is similarly expected to undergo detoxification following reversible binding to GSH. Glutathione levels are unchanged in tissue homogenates following acute exposures but represent a possible adaptive response that may be location-specific and changed with prolonged exposure. For example, repeated exposure to

formaldehyde (18.45 mg/m^3, 6 hrs/d for 9 days) did not affect either the GSH levels or the specific activities of ADH3 and ALDH2 in the nasal mucosa F344 rats (Casanova-Schmitz et al., 1984a). Interfacial DNA levels can be increased by glutathione depletion. This was tested by Casanova and Heck (1987) by exposing rats for 3 hours on two consecutive days to a range (1.11−12.3 mg/m^3) of formaldehyde by inhalation, on Day 1 to nonlabeled formaldehyde and on Day 2 to a mixture of [^{3}H] and [^{14}C]-labeled formaldehyde. Two hours before the exposure on the second day, the animals were injected i.p. with 300 mg/kg phorone, a GSH depleting agent. The authors reported a 90−95% decrease in GSH levels and significant decrease in metabolic incorporation in nasal respiratory and olfactory mucosa and bone marrow of phorone-treated rats. In contrast, the ^{3}H/^{14}C ratios of IF DNA were increased in a concentration-dependent manner for both phorone-treated and control groups of rats, albeit the levels were slightly higher in phorone-treated rats compared to control rats. Thus, depletion of GSH appeared to result in more unmetabolized formaldehyde available for covalent binding (crosslink formation) following 3-hour exposure.

Specific uncertainties regarding the potential impact of endogenous formaldehyde

Since formaldehyde is produced through normal cellular metabolism, several uncertainties exist which might impact the metabolism of exogenous formaldehyde in the body. This section covers the sources of endogenous formaldehyde, comparisons about its concentration gradient, its metabolism and reactivity, and the impact of inhaled formaldehyde on endogenous formaldehyde.

Sources of endogenous formaldehyde

Formaldehyde is endogenously produced through normal cellular metabolism from three main sources. As detailed below and outlined in Figure A-8, these sources include: (1) enzymatic reactions, (2) nonenzymatic reactions, and (3) as a metabolic byproduct of cellular metabolism of xenobiotics (e.g., drugs, environmental contaminants) that enter the body.

(1) Enzymatic pathways that generate formaldehyde endogenously as a normal component of cellular metabolism include four metabolic pathways: methylamine deamination, choline oxidation, histone lysine demethylation, and amino acid metabolism (serine, glycine, methionine). Formaldehyde can also be generated through endogenous generation from exogenous sources (e.g., methanol). These enzymatic sources are summarized in Figure A-8.

Methylamine is endogenously produced through amine catabolism, which upon deamination carried out by the enzyme semicarbazide-sensitive amino oxidase (SSAO) gives rise to formaldehyde. Choline oxidation is another endogenous metabolic process by which formaldehyde is generated. Choline is converted to glycine through several intermediary steps (choline → betaine → dimethylglycine (DMG) → sarcosine → glycine. The last two steps in this pathway are catalyzed by dimethylglycine dehydrogenase (DMGDH) and sarcosine dehydrogenase (SDH), respectively, using flavin adenine dinucleotide (FAD) as a cofactor. During these two steps the dehydrogenases nonenzymatically condense tetrahydrofolate (THF) with formaldehyde generating 5, 10-methylene-THF (5, 10-CH2-THF), also known as "active formaldehyde."

The other mechanism of endogenous formaldehyde production is through histone lysine demethylation, which is carried out by two classes of enzymes near the nucleus in a cell. One is a FAD-dependent amine oxidase, also known as lysine-specific demethylase 1 (LSD1/KDM1). The other one belongs to the Jumonji C terminal (JmjC) domain-containing histone demethylase (JHDM1/KDM2A). The LSD1 and JHDM1 enzymes act, respectively, on dimethyl lysine and trimethyl lysine converting them to monomethyl- and dimethyl lysine with the liberation of formaldehyde as an intermediary product (Shi et al., 2004). Formaldehyde can also be generated from methanol by either enzymatic or nonenzymatic pathways.

(2) Formaldehyde can also be formed nonenzymatically by the spontaneous reaction of methanol with hydroxyl radicals, wherein intracellular hydrogen peroxide is converted to the hydroxyl radical through the Fenton reaction (Cederbaum and Qureshi, 1982). Another mechanism of nonenzymatic production of formaldehyde is through lipid peroxidation of polyunsaturated fatty acids (PUFA) (Shibamoto, 2006; Slater, 1984). It is known that a certain level of oxidative stress and lipid peroxidation occurs in every individual, and these oxidative processes are likely to contribute to endogenous formaldehyde production (Ozen et al., 2008; Zararsiz et al., 2006).

(3) Formaldehyde may also be produced intracellularly during microsomal cytochrome P450 enzyme-catalyzed oxidative demethylation of *N*-, *O*-, and *S*-methyl groups of xenobiotics (ATSDR, 2008) that enter the body through dietary, environmental, or medicinal exposures, as shown in Figure A-8. Dhareshwar and Stella (2008) estimated that formaldehyde released from prodrugs is ≈2−100 mg. However, the authors point out that in humans with endogenous blood levels of ≈2−3 µg/g of blood total formaldehyde (Heck et al., 1985), the fraction of formaldehyde released from xenobiotics may contribute a small fraction to the endogenous pool (Dhareshwar and Stella, 2008).

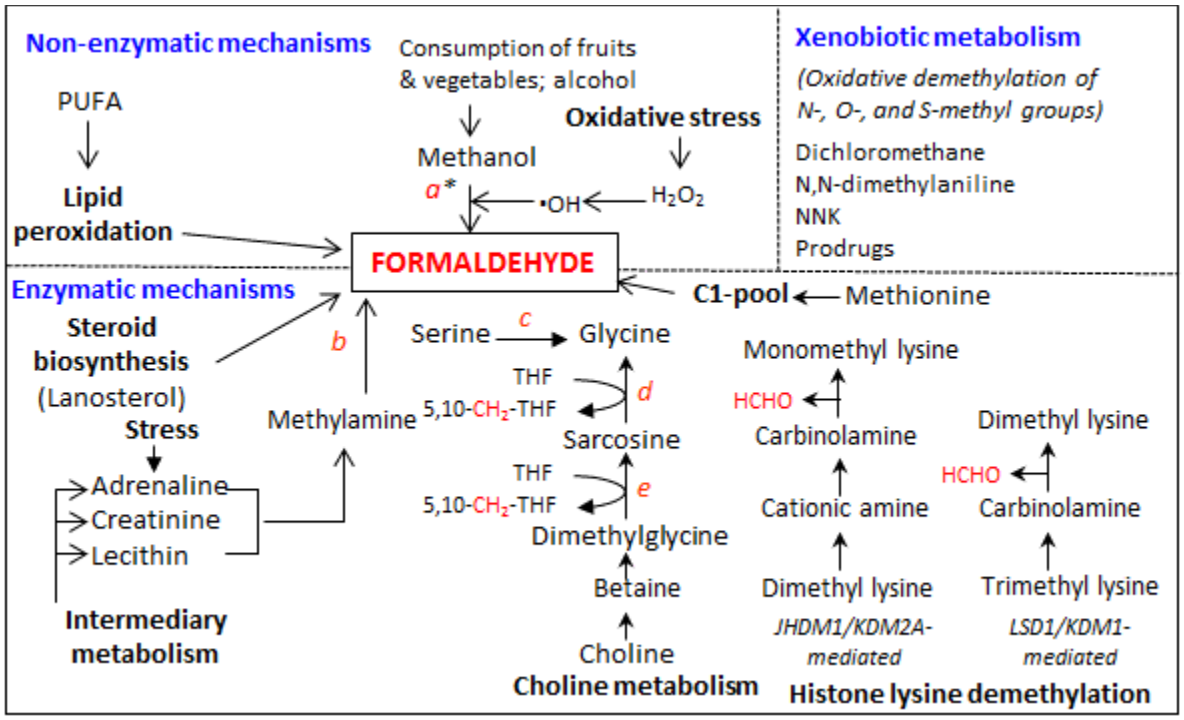

Figure A-8. Endogenous and dietary sources of formaldehyde production.

Formaldehyde is generated in the body through (a) Enzymatic mechanisms - involving (i) Steroid biosynthesis – from lanosterol, (ii) Intermediary metabolism – from methylamine (Yu and Zuo, 1996), (iii) Choline metabolism (Binzak et al., 2000), (iv) Stress – through adrenaline (Yu et al., 1997), (v) histone lysine demethylation (Shi et al., 2004) and (vi) Methanol metabolism (enzymatic) (Skrzydlewska, 2003); (b) Nonenzymatic mechanisms – (i) Methanol oxidation (Cederbaum and Qureshi, 1982) (ii) Lipid Peroxidation of polyunsaturated fatty acids or PUFA (Shibamoto, 2006) and (iii) Oxidative Stress (Slater, 1984); (c) Xenobiotic metabolism – demethylation of chemicals (ATSDR, 2008) and prodrugs (Dhareshwar and Stella, 2008).

Abbreviations: DMG: dimethyl glycine; C1: one carbon; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; THF: tetrahydrofolate; LSD1/KDM1, lysine (K)-specific demthylase 1; JHDM1/KDM2A, JumonjiC-domain containing histone demthylase 1.

Enzymes: *a*, alcohol dehydrogenase-1 (ADH1) in primates and ADH1 and catalase in rodents; *b*, semicarbazole-sensitive amine oxidase; *c*, serine hydroxymethyl transferase; *d*, sarcosine dehydrogenase; *e*, dimethylglycine dehydrogenase.

The presence of comparatively high levels of endogenous formaldehyde in cells of the URT presents an important uncertainty to evaluating the toxicokinetics of inhaled formaldehyde. Once inhaled formaldehyde interacts with aqueous matrices such as mucus and is hydrated, the biochemical interactions of inhaled formaldehyde and endogenous formaldehyde are assumed to be very similar, given that there are no differences in chemical structure. However, other than in the nucleus (i.e., the experiments detailing DNA adducts), no data are available to inform where and to what extent endogenous and exogenous formaldehyde may be available to participate in these reactions.

Although much is unknown regarding the impact of endogenous formaldehyde on the formaldehyde uptake and metabolism as outlined in the sections above, uncertainties relevant to

interpreting the potential for biological differences between inhaled formaldehyde and endogenous formaldehyde are important to specify. Several of these uncertainties, which are essential to consider when comparing the distribution and macromolecular binding of endogenous formaldehyde versus inhaled formaldehyde, are outlined below.

Comparisons regarding the concentration gradient of endogenous formaldehyde

Endogenous formaldehyde is known to be produced within all cells of the URT. The specific levels of endogenous formaldehyde within each type of cell, or even within the various components of the nasal tissue (e.g., the respiratory mucosa lining the maxilloturbinates; the squamous epithelium lining the luminal surface of the nasal vestibule), are likely to vary across individuals and have not been experimentally defined. However, there is likely to be a general level (for which estimates have been calculated) that could be applied homogenously across the URT tissue. With formaldehyde inhalation, it does not appear that the general (endogenous) levels of formaldehyde in the entire nasal mucosa are significantly altered (e.g., e.g., Heck et al., 1983; Heck et al., 1982). A concern is raised when interpreting observed changes in the levels or macromolecular binding of endogenous formaldehyde, as compared to those caused by inhaled formaldehyde. Specifically, a consideration of the tissue region assayed needs to be incorporated. While endogenous formaldehyde is produced within all regions of the nasal mucosa, uptake of inhaled formaldehyde occurs at specific anatomic locations, primarily the squamous epithelium and respiratory mucosa in anterior regions of the nose. Thus, comparisons of endogenous levels (or effects) in homogenates containing isolates where all components are "target" tissues versus inhaled formaldehyde levels (or effects) in homogenates containing both "target" and "nontarget" (e.g., olfactory epithelium) isolates are difficult to interpret. Notably, the comparisons involving N^2-hm-dG DNA adducts (Lu et al., 2011; Moeller et al., 2011; Lu et al., 2010a) addressed this concern. These authors compared isolates of nasal respiratory mucosa and observed that dose-dependent increases in N^2-hm-dG adducts due to short-term, exogenous exposure do not reach the level of N^2-hm-dG adducts due to endogenous formaldehyde until exposure to >11 mg/m^3 formaldehyde (Lu et al., 2011); relatedly, low levels of dG-CH_2-dG adducts appeared to be higher with exogenous exposure to 12.3 mg/m^3 formaldehyde for 5 days, as compared to adducts caused by endogenous formaldehyde (Lu et al., 2010a). Similarly, the measurements by Heck et al. (1983; 1982) also appeared to quantify these effects based on isolated respiratory mucosa.

A related concern, based on the decreasing concentration of inhaled formaldehyde reaching deeper components of the nasal mucosa, is that exogenous formaldehyde is not expected to interact to the same extent with all components (cellular and extracellular) of the nasal mucosa. Rather, these interactions are highly enriched in the epithelial cells and associated cellular/extracellular components along the apical surface of the respiratory mucosa. This is assumed to be in contrast with endogenous formaldehyde, which is present (possibly at comparable levels) inside all cells of the nasal mucosa. Although the respiratory epithelium would be expected to comprise the majority of the cellular makeup of the isolated mucosa, contributions from cells in the *lamina propria* to

measured levels and effects of endogenous formaldehyde would be expected to far outweigh those same contributions attributable to exogenous exposure. Thus, this introduces an uncertain amount of inequality to comparisons of the relative contributions of exogenous and endogenous formaldehyde to macromolecular binding. It also highlights an important characteristic of the levels of exogenous and endogenous formaldehyde in tissue isolates; namely, that these levels do not necessarily reflect, nor even approximate, the comparative levels in the target cells. However, it would be methodologically arduous to isolate select portion(s) of the respiratory mucosa for comparison, and as such, it does not appear that any studies have done so.

Comparisons regarding metabolism and reactivity of endogenous formaldehyde

As compared to exogenous formaldehyde, for which it is unknown how quickly it may be detoxified by the normal cellular machinery, the production and subsequent detoxification of endogenous formaldehyde appears to be kept under strict control. As mentioned earlier, the majority of endogenous formaldehyde is reversibly bound to GSH at any time (Sanghani et al., 2000).

The regulation of endogenous formaldehyde appears to be imperfect, given the presence of endogenous N^2-$HOCH_2$-dG (dG) adducts (Swenberg et al., 2011). The endogenous adduct levels reported by Swenberg et al. (2011) are about the same as the exogenous levels that would result from a single 6-hour exposure to ≈10 ppm formaldehyde. Given that endogenous formaldehyde is present continuously, the equivalent continuous exposure to exogenous formaldehyde that would result in the same dG levels must be somewhat less than 10 ppm, perhaps 1 or 2 ppm (i.e., a continuous exposure to 2 ppm could produce the same dG levels as a single, 6-hour exposure to 10 ppm; a much more detailed pharmacokinetic analysis would be required to exactly determine the exact equivalent exposure). Toxicokinetic models that are calibrated or matched with formaldehyde-induced DPX data *and* use the DNA-binding constant determined in vitro by Heck and Keller (1988) can be used with reasonable reliability to predict induced tissue levels of formaldehyde in the rat nose from exogenous exposure. For example, Georgieva et al. (2003) predict an exogenous level in nasal tissue of around 17 µM from a 6-ppm exposure. Heck et al. (1982) reported a total endogenous level in rat nasal tissue of 12.6 µg/g or 420 µM. But as described just above, the dG adducts from endogenous formaldehyde correspond to an exposure of less than 10 ppm, though the total amount of endogenous formaldehyde is over 20-times higher. Hence, much, but not all, of the endogenous formaldehyde (measured by Heck et al. (1982)) must be bound or sequestered in a way that reduces its ability to react with DNA, in comparison with exogenous formaldehyde.

Impact of inhaled formaldehyde on the function of endogenous formaldehyde

Although formaldehyde inhalation does not appear to result in a measurable change in the total level of formaldehyde in the nasal tissue of rats (Heck et al., 1982), it has yet to be determined whether exposure results in any changes to the normal functions of endogenous formaldehyde. For

example, in the study by Lu et al. (2011), rats exposed to ^{13}C-formaldehyde showed a concentration-dependent increase in the exogenous hm-dG adduct levels, and the corresponding endogenous N^2-hm-dG adduct levels were highly variable at different exposure concentrations in the nasal tissues. In addition to the potential "compartmentalization" differences mentioned above, the endogenous DNA adduct levels, reflective of endogenous formaldehyde, do not appear to be static. Possible effects of exogenous formaldehyde exposure on metabolism and distribution processes of endogenous formaldehyde cannot be conclusively ruled out. However, no appreciable changes in the number of adducts formed as a result of interactions of endogenous formaldehyde with cellular constituents have been noted, even in the presence of formaldehyde exposure (e.g., e.g., Yu et al., 2015b).

Summary of potential modifying factors and specific uncertainties

The toxicokinetics of formaldehyde may be influenced by certain formaldehyde-related effects, such as mucociliary clearance (Morgan et al., 1983), reflex bradypnea (rodents only) and reduction in minute volume (Chang et al., 1983; Chang et al., 1981), and dynamic tissue remodeling (Kamata et al., 1997), which have the potential to modulate formaldehyde uptake and clearance. For example, during repeated inhalation exposure to formaldehyde, mice but not rats lower their minute volume thereby restricting the intake of the gas (Chang et al., 1983; Chang et al., 1981), which may impact dosimetric adjustment if extrapolated to humans. Exposure to formaldehyde can also cause a perturbation of ADH3-dependent pathways involved in cell proliferation (Nilsson et al., 2004; Hedberg et al., 2000), protein modification and cell signaling (Que et al., 2005), GSNO metabolism, and deregulation of nitric oxide-dependent pathways (Thompson et al., 2010). In rats exposed by inhalation to formaldehyde, a rapid GSH depletion can result in more free formaldehyde available for covalent binding and lowering metabolic incorporation (Casanova and Heck, 1987).

A.2.5. Conclusions Regarding the Toxicokinetics of Inhaled Formaldehyde Within the POE

Within the POE, a majority of inhaled formaldehyde is rapidly retained in the URT of humans and experimental animals, irrespective of species differences in the anatomy, physiology, and breathing patterns. Based on formaldehyde's molecular and biochemical properties, it can reasonably be inferred that total formaldehyde levels are not significantly affected by exogenous exposure. Also, one can conclude that following inhalation, formaldehyde levels are successively reduced as formaldehyde from the air penetrates through the various components of the nasal mucosa. Formaldehyde levels are reduced through interactions with components of the mucus and through mucociliary clearance; through reactions with cellular materials at the plasma membrane of the respiratory epithelium; via interactions with glutathione (GSH) and other macromolecules in the intracellular and extracellular space; through localized metabolism and conjugation reactions; and through reversible interactions with intracellular materials. This results in the formation of a gradient of formaldehyde across the tissue space, with the greatest formaldehyde concentration at the apical surface of the mucosa, and the lowest levels of formaldehyde at deeper components of

the tissue, such as the nasal associated lymphoid tissues (NALT) and blood vessels. In the URT, formaldehyde is metabolized by cytosolic ADH3 (major) and mitochondrial ALDH2 (minor) enzymes to formate which is further metabolized to CO_2 and eliminated in expired air, enters the 1C pool leading to metabolic incorporation, or is excreted in urine unchanged. The toxicokinetics of formaldehyde may be influenced by several modifying factors in the nasal passages, which should be considered for dosimetric adjustment when extrapolating to humans.

A.2.6. Toxicokinetics of inhaled formaldehyde beyond the portal of entry

Consistent with the previously described concentration gradient of inhaled formaldehyde within the POE, multiple studies report that very little inhaled formaldehyde reaches the vasculature of the respiratory tract to allow for absorption into the systemic circulation. Similarly, there is very little evidence that inhaled formaldehyde is distributed to tissues such as the bone marrow, liver, or brain. Studies examining the potential for direct interactions of inhaled formaldehyde with cellular macromolecules at distal sites have also not reported any evidence of these effects, despite observing that endogenous formaldehyde elicits such effects. Although the evidence is not entirely conclusive, and some uncertainties remain to be explored, the currently available data support an overall conclusion that appreciable amounts of inhaled formaldehyde are not distributed outside of the URT. Formaldehyde produced endogenously through enzymatic and nonenzymatic mechanism as well as that produced by the demethylation of xenobiotics (ATSDR, 2008), may pose some uncertainties for the exogenous formaldehyde metabolism.

A.2.7. Levels of Endogenous and Inhaled Formaldehyde in Blood and Distal Tissues

Using the detection methods employed by Heck et al. (1982), two studies from the same group reported endogenous levels of total formaldehyde in blood to be 2.61 ± 0.14 μg/g of blood in unexposed human subjects (Heck et al., 1985), 2.24 ± 0.07 and 2.71± 0.29 μg/g of blood in control F344 (Heck et al., 1985) and SD rats (Kleinnijenhuis et al., 2013), respectively, and 2.42 ± 0.09 μg/g of blood in unexposed rhesus monkeys (Casanova et al., 1988), providing relatively consistent measurements across species with an average blood level of ≈2.5 μg/g (≈0.1 mM) (see Table A-11). Levels of endogenous formaldehyde higher than in blood were also detected in other distal tissues of rats, although the nasal tissue contained the highest levels (Heck et al., 1982). The blood formaldehyde levels were not significantly changed when tested during exposure or shortly after exposure to formaldehyde concentrations ranging from 2.3 to 7.4 mg/m^3 across the three species, with varying durations of exposure (Casanova et al., 1988; Heck et al., 1985). The lack of increase in the blood formaldehyde levels could also be due to the metabolism of formaldehyde in human erythrocytes, which are known to contain the formaldehyde metabolizing enzymes ADH3 (Uotila and Koivusalo, 1987) and ALDH2 (Inoue et al., 1979).

The tissue levels of endogenous formaldehyde determined experimentally by Heck et al. (1982) may be highly uncertain. Campbell Jr. (2020) assessed these values to be 20× lower based upon their modeling estimates and attributed this discrepancy to the potential for the Heck et al.

measurement methodology to overestimate tissue formaldehyde levels. This is addressed again in Section A.2.12 in a discussion of model derived estimates of the effects of endogenous formaldehyde on formaldehyde dosimetry.

EPA notes that while these data indicate that inhaled formaldehyde is not absorbed into the systemic circulation, a rough bounding calculation based on the human data indicates that the Heck et al. (1985) experiment lacks the sensitivity needed to reach this conclusion. This bounding calculation assumes that the 2.3 mg/m^3 of inhaled formaldehyde completely mixes with the blood, and because of its high solubility, it has a volume of distribution equal to that of all body water [0.57 L/kg of body weight; (Guyton, 1991)]. Using these parameters, the Heck et al. (1985) experiment is estimated to result in an increased blood formaldehyde concentration of 0.016 µg/g[2]. This quantity is one-half the experimental error of 0.03 µg/mL. Hence, even if all of the 2.3 mg/m^3 of inhaled formaldehyde completely mixes with the blood, under the experimental protocol above for the human exposure, formaldehyde blood concentration would increase by 0.016 µg/g, a quantity that cannot be detected by the Heck et al. (1985) experiment.[3] Moreover, this quantity is two orders of magnitude lower than the endogenous blood levels. Hence, these results are consistent with a lack of ^{14}C radiolabel increases in the plasma of rats exposed to ^{14}C formaldehyde (Heck et al., 1983), as well as a lack of increase in total formaldehyde calculated following exposure of rats to ^{13}C formaldehyde (Kleinnijenhuis et al., 2013). Altogether, the data argue that the amount of inhaled formaldehyde absorbed into the blood is not likely to be significant, even if one assumes that only 5% of the endogenous formaldehyde in blood is not sequestered.

A similar trend was observed in distal tissues. Heck et al. (1983) exposed rats to a range of ^{14}C-formaldehyde concentrations (6.14–29.48 mg/m^3 for 6 hours) and observed that the ratio of tissue distribution relative to plasma radioactivity (µmole equivalents/g tissue) was not correlated with the exposure concentration, except in the esophagus (Heck et al., 1983). Mucociliary transport from the nose and trachea may have led to these relatively higher esophageal levels. Overall, these data also indicate that tissue distribution of formaldehyde levels were independent of the exposure concentration and duration of exposure.

Overall, the published data demonstrate no significant increase in formaldehyde levels in blood following formaldehyde inhalation. These data also report no significant differences in tissue and blood formaldehyde levels between preexposed and naïve animals. Such observations were obtained from short-term experimental animal studies based on ^{14}C-radiolabeling by GC-MS. The use of only this approach is problematic because there is no distinction as to whether the

[2]Heck et al. (1985) air concentration = 1.9 ppm = 1.9*1.23 mg/m^3 = 2.34 mg/m^3; t = 40/60 h; Inhalation Rate = 10–15 cubic m/day. Assuming 10 m^3/24 hrs, we get 10/24 m^3/h. Formaldehyde inhaled = 1.9 × 1.23 × (10/24) × 40/60 h = 0.649 mg. Body water = 40 kg for a 70-kg man (Guyton, 1991); concentration of HCHO = HCHO inhaled/body water in mg/kg = 0.649/40 = 0.0162 mg/kg or µg/g.

[3]Even if one were to assume that formaldehyde stays only in the blood stream, this concentration increases to 0.12 µg/g of blood, which is still within the experimental error.

formaldehyde measured in these studies is free, reversibly or irreversibly bound, measured as formate, or part of the one-carbon pool. Nevertheless, taken together with the bounding calculations and relative activity calculations described above, the lack of significance of exogenous formaldehyde reaching distal tissues appears to hold even given the uncertainty.

Table A-11. Summary of blood and tissue levels of total[a] formaldehyde in humans and experimental animals following inhalation exposure to formaldehyde

Reference and species	Exposure and analysis	Observations		
Heck et al. (1985) Human volunteers Male, *n*=4; female, *n*=2 24–44 yrs old	2.34 ± 0.07 mg/m^3 CH_2O (**source not specified**); 40 min exposure in a walk-in chamber; venous blood collected before and after exposure; Total CH_2O measured as PFPH derivative by GC-MS/SIM	*Total[a] formaldehyde (µg/g of blood)*		
		Before exposure: After exposure:	2.61 ± 0.14 2.77 ± 0.28	
Casanova et al. (1988) Monkeys, rhesus Male, *n*=4; 200–250 g	7.37 mg/m^3 CH_2O (from PFA); 6 hrs/d, 4 d/wk, 4 wks; chamber inhalation; whole-body exposure; pre- and postexposure blood collected; Total CH_2O measured as PFPH derivative by GC-MS/SIM	*Total[a] formaldehyde (µg/g of blood)*		
		Before exposure: 0 min. after exposure 40 min. after exposure:	2.42 ± 0.09 1.84 ± 0.15 2.04 ± 0.40	
Heck et al. (1985) Rats, Fischer Male, *n*=4, 232 ± 22 g	17.69 ± 2.95 mg/m^3 CH_2O (**source not specified**); 2-hrs exposure; chamber inhalation; nose-only; controls–no exposure; Total CH_2O measured as PFPH derivative by GC-MS/SIM	*Total[a] formaldehyde (µg/g of blood*		
		Before exposure: After exposure:	2.24 ± 0.07 2.50 ± 0.07	
Kleinnijenhuis et al. (2013) Rats, Sprague Dawley Male, *n*=10 12 wks-old	12.3 mg/m^3 $^{13}CH_2O$ (19.3% in aqueous solution: **source not specified**); 6-hrs exposure, Nose-only chamber; Blood samples collected before, during and after exposure; analyzed by HPLC-MS/MS after derivatizing with 2,4-DNPH	*Total[a] formaldehyde (mg/L of blood[b])*		
		Before Exposure: During Exposure (3 hrs): During Exposure (6 hrs): After Exposure (~6.2 hrs): After Exposure (6.5 hrs):	2.71 ± 0.29 2.63 ± 1.12 2.01 ± 0.48 2.11 ± 0.35 1.81 ± 0.22	
Heck et al. (1982) Rats, Fischer Male, *n*=8 200–250 g	7.37 mg/m^3 $^{13}CH_2O$ from PFA; 6 hrs/d; 10-days exposure; chamber inhalation; CH_2O measured as PFPH derivative by GC/MS	**Rat tissue levels (mean ± SE) of total[a] CH_2O**		
			Unexposed	*Exposed*
		Tissue	µg/g	µg/g
		Nasal mucosa	12.6 ± 2.7	11.7 ± 3.6
		Liver	6.03 ± 0.5	NR
		Testes	8.40 ± 3.0	NR
		Brain	2.91 ± 0.42	NR
Heck et al. (1983) Rats, Fischer Male, *n*=3; 180–250 g	Two groups: (a) *preexposure*; (b) *naïve*; On days 1–9: group a) received 18.42 mg/m^3; CH_2O (from PFA); whole body exposure, 6 hrs/d; group b): no exposure. On day 10: groups a and b received ^{14}C-CH_2O (from PFA) for 6 hrs, nose-only exposure. Tissue homogenates counted	**Animals Exposed**	**Equivalents of ^{14}C in tissues (Mean ± SE)**	
		naïve rats	**Nasal mucosa**	**Plasma**
		preexposed	2148 ± 255	76 ± 11

Reference and species	Exposure and analysis		Observations		
	with LSC for $^{14}CO_2$ trapped in ethanolamine in 2-methoxy-ethanol counted for radioactivity			2251 ± 306	79 ± 7
				Not significant	Not significant
Heck et al. (1983) Rats, Fischer, Male, *n*=12	Naïve rats: dosed with 6.14, 12.28, 18.42 or 29.48 mg/m^3 ^{14}C-CH_2O (from PFA); 6-hrs nose-only; sacrificed immediately after exposure; tissue homogenates counted with LSC.	**Tissue**	**(DPM/g tissue)/(DPM/g plasma)[c]**	**Tissue**	**(DPM/g tissue)/(DPM/g plasma)[c]**
		Esophagus	4.94 ± 1.23	Spleen	1.59 ± 0.50
		Kidney	3.12 ± 0.47	Heart	1.09 ± 0.09
		Liver	2.77 ± 0.25	Brain	0.37 ± 0.06
		Intestine	2.64 ± 0.48	Testes	0.31 ± 0.05
		Lung	2.05 ± 0.36	RBC	0.30 ± 0.08

[a]Includes free and reversibly bound formaldehyde (Heck et al., 1982).
[b]Calculated concentration in blood and corrected for stability.
[c]Values (Mean ± SD) are ratios of concentrations (radioactivity) in tissues relative to plasma immediately after a 6-hour exposure to ^{14}C-formaldehyde averaged for four concentration groups (*n* = 12/concentration).
CH_2O, formaldehyde; GC, gas chromatography; LC, liquid chromatography; MS, mass spectrometry; HPLC-MS/MS, high performance liquid chromatography/tandem mass spectroscopy; PFA, paraformaldehyde; SIM, selected ion monitoring; DNPH, dinitrophenyl hydrazine; PFPH, pentafluorophenyl hydrazine; DPM, disintegrations per minute; ND, not detected; UPLC, ultraperformance liquid chromatography; $NaCNBH_3$, sodium cyanogen borohydride.

Covalent binding of formaldehyde to macromolecules beyond POE

Formaldehyde has been shown to interact with the macromolecules in the blood or blood cells, but not in other distal organs as described below.

Evidence of covalent binding of formaldehyde to blood proteins

Formaldehyde has also been shown to covalently bind to serum proteins such as the amino acid valine in hemoglobin (Hb) forming N-methylvaline adducts in workers in plywood and laminate factory workers with occupational exposure (Bono et al., 2006). Also, with human serum albumin (HSA) it forms formaldehyde-HSA complexes (Thrasher et al., 1990). However, N^6-formyllysine, another formaldehyde-induced protein adduct that also occurs endogenously, was not detectable in blood cells or in distal tissues (liver, lung, and bone marrow) in rats exposed to exogenous ^{13}C-labeled formaldehyde (Edrissi et al., 2013a).

Evidence of DPX in the blood cells of formaldehyde exposed workers

DPXs have also been reported in the peripheral blood lymphocytes (PBLs) of formaldehyde-exposed workers (Shaham et al., 2003; Shaham et al., 1997; Shaham et al., 1996). Shaham et al.

(1996) observed a statistically significant increase in DPX levels in PBLs compared to unexposed subjects and reported a linear relationship between years of exposure and the amount of DPX.

Lack of experimental evidence of endogenous and exogenous DNA monoadducts and DNA-protein crosslinks in blood and distal tissues

According to the available adduct studies, inhaled formaldehyde does not reach systemic tissues in concentrations sufficient to elicit detectable interactions of formaldehyde with DNA. In the bone marrow of monkeys (Moeller et al., 2011), and in the bone marrow, liver, lung, spleen, thymus, and blood of rats (Lu et al., 2010a), DNA monoadducts were formed by interactions with endogenous formaldehyde, but adducts formed from exogenous formaldehyde were not found (see Table A-12). It is important to note that Moeller et al. (2011) observed 6–8 times higher endogenous N^2-hm-dG adducts in the bone marrow compared to the nasal tissues of monkeys. Although there were some limitations with the experimental methods, including a possible overestimation of endogenous adducts due to reasons discussed (see Section A.2.3), the data support a general lack of systemic distribution of inhaled formaldehyde.

As described for the POE tissues, efforts have been made to differentiate covalent binding from metabolic incorporation in bone marrow. Male F344 rats were exposed to a mixture of ^{3}H- and ^{14}C-labeled formaldehyde for 6 hours at 0.37–18.42 mg/m^3 1 day after exposure to nonradioactive formaldehyde with the same exposure range (Casanova-Schmitz et al., 1984b). The authors extracted IF DNA from bone marrow (femur) and determined the ^{3}H/^{14}C ratios of different phases of DNA (i.e., AQ DNA and IF DNA). As previously described, a sample that contains adducts and crosslinks should be higher than in a sample that primarily contains metabolically incorporated formaldehyde. In contrast to results in respiratory mucosa, bone marrow from the distal femur did not show increased ^{3}H/^{14}C ratio in the IF DNA or AQ DNA or proteins phase as a function of formaldehyde concentration (see Figure A-9). Therefore, the authors concluded that radiolabeled metabolites of formaldehyde reached the distal site (femur bone marrow) and were subsequently metabolically incorporated into macromolecules (see Figure A-7). In total, these data suggest that the labeling of bone marrow macromolecules was likely due to metabolic incorporation rather than due to covalent binding (Casanova-Schmitz et al., 1984b).

Recently Lai et al. (2016) developed an ultrasensitive mass spectrometry method which distinguishes unlabeled DPX from $^{13}CD_2$-labeled DPXs induced respectively, from endogenous and exogenous formaldehyde. The authors demonstrated that inhalation exposure of stable isotope labeled ($^{13}CD_2$) formaldehyde to rats (18.45 mg/m^3; 6 hours/day; 1–4 days) and monkeys (2.5 mg/m^3; 6 hours/day; 2 days) induced exogenous DPX in POE tissues such as nasal passages in both species, but not in distal tissues, such as bone marrow and peripheral blood monocytes (rats and monkeys) and liver (monkeys), although endogenous DPX were detectable in all tissues (see Table A-13). These observations further confirm the lack of experimental evidence of formaldehyde distribution to distal tissues.

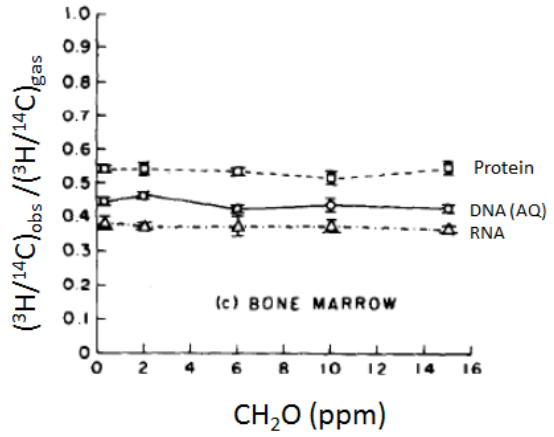

Figure A-9. $^3H/^{14}C$ ratios in macromolecular extracts from rat bone marrow following 6-hour exposure to ^{14}C- and ^3H-labeled formaldehyde (0.3, 2, 6, 10, and 15 ppm, corresponding to 0.37, 2.46, 7.38, 12.3, 18.42 mg/m^3, respectively)**.**

Source: Adapted from Casanovaschmitz et al. (1984)

Table A-12. Summary of endogenous and exogenous DNA monoadducts in distal tissues of monkeys and rats following inhalation exposure of $^{13}CD_2$-labeled formaldehyde

Reference and design	Exposure and analysis[a]		CH_2O conc.	Observations	
Moeller et al. (2011); Monkeys, cynomolgus; n = 3	2.3 and 7.5 mg/m^3 [$^{13}CD_2$]-CH_2O from PFA; 6 hrs/d; for 2 d; whole-body exposure; sacrificed immediately after exposure; necropsied within 3 hrs; nasal mucosa and bone marrow collected; tissue DNA extracted, reduced with $NaCNBH_3$, digested and analyzed by nano-UPLC/MS.		(mg/m^3)	Bone marrow	
				Endogenous adducts	Exogenous adducts
				DNA adducts/10^7dG	
			2.34	17.5 ± 2.6	ND
			7.5	12.4 ± 3.6	ND
Yu et al. (2015b); Monkeys, cynomolgus;	0 (air control), 2.4 or 7.5 mg/m^3 [$^{13}CD_2$]-CH_2O from [$^{13}CD_2$]PFA; nose-only exposure; 6 hrs/d for 2 consecutive days; Sacrificed immediately after exposure; Tissue DNA was extracted, reduced with $NaCNBH_3$, digested and analyzed by nano-UPLC-MS/MS	**Distal tissue**		N^2-hm-dG/10^7 dG	
		Scrapped bone marrow (Animal#1)	2.4	17.5 ± 2.6	ND
		Scrapped bone marrow (Animal#2)	7.5	12.4 ± 3.6	ND
		Air control (Animal#2)	0	10.18 ± 1.35	ND
		Scrapped bone marrow (Animal#2)	7.5	11.00 ± 2.01	ND
		Air control (Animal#2)	0	5.65 ± 2.12	ND
		Saline extrusion bone marrow (Animal#2)	7.5	4.41 ± 1.00	ND
		Air control (Animal#2)	0	3.64 ± 1.09	ND
		White blood cells (Animal#2)	7.5	3.79 ± 1.19	ND
		Adduct →	**N^2-hm-dG/10^7 dG[a]**		

Reference and design	Exposure and analysis[a]			CH_2O conc.	Observations	
Lu et al. (2010a); Rats, Fisher; Male, n=5-8	12.3 mg/m^3 [$^{13}CD_2$]-CH_2O from [$^{13}CD_2$]PFA; 6 hrs/d, 1 or 5 d; nose-only exposure; Sacrificed immediately after exposure. Lung, liver, spleen, bone marrow, thymus, and blood collected; tissue DNA extracted, reduced with $NaCNBH_3$, digested and analyzed by nano-UPLC-MS/MS	Duration→	1 day		5 days	
		Tissue	*Endogenous*	*Exogenous*	*Endogenous*	*Exogenous*
		Lung	2.39 ± 0.16[b]	ND[c]	2.61 ± 0.35	ND
		Liver	2.66 ± 0.53	ND	3.24 ± 0.42	ND
		Spleen	2.35 ± 0.31	ND	2.35 ± 0.59	ND
		Bone marrow	1.05 ± 0.14	ND	1.17 ± 0.35	ND
		Thymus	2.19 ± 0.36	ND	1.99 ± 0.30	ND
		Blood[d]	1.28 ± 0.38	ND	1.10 ± 0.28	ND
		Adduct →	**N^6-hm-dA/10^7 dA[a]**			
		Duration→	1 day		5 days	
		Distal Tissue	*Endogenous*	*Exogenous*	*Endogenous*	*Exogenous*
		Lung	2.62 ± 0.24	ND	2.47 ± 0.55	ND
		Liver	2.62 ± 0.46	ND	2.87 ± 0.65	ND
		Spleen	1.85 ± 0.19	ND	2.23 ± 0.89	ND
		Bone marrow	2.95 ± 1.32	ND	2.99 ± 0.08	ND
		Thymus	2.98 ± 1.11	ND	2.48 ± 0.11	ND
		Blood[d]	3.80 ± 0.29	ND	3.66 ± 0.78	ND
		Adduct →	**dG-CH_2-dG/10^7 dG[a]**			
		Duration→	1 day		5 days	
		Distal Tissue	*Endogenous*	*Exogenous*	*Endogenous*	*Exogenous*
		Lung	0.20 ± 0.04[e]	ND	0.20 ± 0.03	ND
		Liver	0.18 ± 0.05	ND	0.21 ± 0.08	ND
		Spleen	0.15 ± 0.06	ND	0.16 ± 0.08	ND
		Bone marrow	0.09 ± 0.01	ND	0.11 ± 0.03	ND
		Thymus	0.10 ± 0.03	ND	0.19 ± 0.03	ND
		Blood[d]	0.12 ± 0.09	ND	0.10 ± 0.07	ND
Yu et al. (2015b); Rats, Fischer;	0 (air control), 2.4 or 7.5 mg/m^3 [$^{13}CD_2$]-CH_2O from [$^{13}CD_2$]PFA; nose-only exposure; 6 hrs/d for 2 consecutive days; Sacrificed immediately after exposure; tissues collected. Tissue DNA	**Formaldehyde exposure duration**	Rat bone marrow		Rat white blood cells	
			N^2-OHMe-dG (adducts/10^7 dG)			
			Endogenous[f]	Exogenous	Endogenous[f]	Exogenous
		Air control	3.58 ± 0.99	ND	2.76 ± 0.66	ND
		7 days	3.37 ± 1.56	ND	2.62 ± 1.12	ND

Reference and design	Exposure and analysis[a]			CH_2O conc.	Observations	
	was extracted, reduced with $NaCNBH_3$, digested and analyzed by nano-UPLC-MS/MS	14 days	2.72 ± 1.36	ND	2.26 ± 0.46	ND
		21 days	2.44 ± 0.96	ND	2.40 ± 0.47	ND
		28 days	3.43 ± 2.20	0.34[g]	2.49 ± 0.50	ND
		28 days + 6 hrs PE	2.41 ± 1.14	ND	2.97 ± 0.58	ND
		28 days + 24 hrs PE	4.67 ± 1.84	ND	2.57 ± 0.58	ND
		28 days + 72 hrs PE	5.55 ± 0.76	ND	1.75 ± 0.26	ND
		28 days + 168 hrs PE	2.78 ± 1.94	ND	2.61 ± 1.22	ND
		Distal tissue	N^2-OHMe-dG (adducts/10^7 dG)			
			Air control		28-day exposure	
			Endogenous	Exogenous	Endogenous	Exogenous
		Thymus	0.78 ± 0.04	ND	0.63 ± 0.06	ND
		TBLN	3.46 ± 1.24	ND	3.01 ± 0.71	ND
		Lymph nodes	2.99 ± 0.85	ND	2.80 ± 1.38	ND
		Trachea	3.18 ± 0.72	ND	2.63 ± 0.92	ND
		Lung	2.29 ± 0.24	ND	2.13 ± 0.26	ND
		Spleen	2.18 ± 0.19	ND	1.83 ± 0.25	ND
		Kidneys	2.17 ± 0.60	ND	1.99 ± 0.09	ND
		Liver	1.97 ± 0.38	ND	1.80 ± 0.02	ND
		Brain	2.13 ± 0.17	ND	2.35 ± 1.00	ND

[a]The limit of detection for dG monoadducts, dA monoadducts, and dG-dG crosslinks was ≈240, ≈75, and ≈60 amol, respectively.

[b]*n* = 4–5 tissues.

[c]Not detectable in 200 µg of DNA.

[d]60–100 µg of DNA was typically used for analysis of white blood cells isolated from blood.

[e]*n* = 3.

[f]No statistically significant difference was found using the 2-sided Dunnett's test (multiple comparisons with a control).

[g]The amount of exogenous N2-hm-dG adducts that was found in only 1 bone marrow sample analyzed by AB SCIEX Triple Quad 6500.

Abbreviations: PFA, paraformaldehyde; UPLC, ultra-pressure liquid chromatography; MS, mass spectrometry; N2-hm-dG, N2-hydroxymethyl-deoxyguanosine; N6-hm-dG, N6-hydroxymethyl-deoxyadenosine; dG-CH2-dG, dG-dG crosslink; TBLN, tracheal bronchial lymph nodes; ND, not detected.

Table A-13. Summary of endogenous and exogenous DNA-protein crosslinks in distal tissues of monkeys and rats following inhalation exposure of $^{13}CD_2$-labeled formaldehyde

Reference and design	Exposure and analysis		Exposure duration	CH_2O conc.	Observations	
Lai et al. (2016); Monkeys, cynomolgus;	0 (air control) or 7.4 mg/m^3 [$^{13}CD_2$]-CH_2O from PFA; 6 hrs/d; for 2 d; whole-body exposure; PBMC, bone marrow and liver collected; tissue DNA extracted; dG-Me-Cys purified on HPLC and analyzed by nano-LC/ESI/MS-MS.	Tissue analyzed		(mg/m^3)	Endogenous adducts	Exogenous adducts
					dG-Me-Cys/10^8 dG	
		PBMC	2 d	0	1.34 ± 0.25	ND
			2 d	7.4	1.57 ± 0.58	ND
		Bone marrow	2 d	0	2.30 ± 0.30	ND
			2 d	7.4	1.40 ± 0.46	ND
		Liver	2 d	0	15.46 ± 1.98	ND
			2 d	7.4	11.80 ± 2.21	ND
Lai et al. (2016); Rats, F344; *N*=4-6.	0 (air control) or 18.5 mg/m^3 [$^{13}CD_2$]-CH_2O from PFA; 6 hrs/d; for 1,2, 4 d; whole-body exposure; PBMC, and bone marrow collected; tissue DNA extracted; dG-Me-Cys purified on HPLC and analyzed by nano-LC/ESI/MS-MS.	Tissue analyzed	Exposure Duration	(mg/m^3)	Endogenous adducts	Exogenous adducts
					dG-Me-Cys/10^8 dG	
		PBMC	4 d	0	4.98 ± 0.61	ND
			1 d	18.5	3.26 ± 0.73	ND
			2 d	18.5	3.00 ± 0.98	ND
			4 d	18.5	7.19 ± 1.73	ND
		Bone marrow	4 d	0	1.64 ± 0.49	ND
			1 d	18.5	1.80 ± 0.47	ND
			2 d	18.5	1.84 ± 0.61	ND
			4 d	18.5	1.58 ± 0.38	ND

Abbreviations: PFA, paraformaldehyde; LC, liquid chromatography; MS, mass spectrometry; HPLC, high performance liquid chromatography; CH_2O, formaldehyde; DPX, DNA-protein crosslinks; dG-Me-Cys, deoxyguanosine-methyl-cysteine; PBMC, peripheral blood mononuclear cell; ESI, electron spray ionization.

A.2.8. Conjugation, Metabolism, and Speciation of Formaldehyde Outside the POE

Were inhaled formaldehyde to reach the blood or distal tissues, the same factors described for POE effects, specifically those regarding metabolism, reactivity, and the role of endogenous formaldehyde, would be relevant to other tissues. The majority of formaldehyde that reached these systemic sites is expected to be in the form of methanediol which is not reactive with macromolecules.

A.2.9. Elimination Pathways of Exogenous and Endogenous Formaldehyde

Elimination pathways of endogenous and exogenous pathways may not be different since all tissues contain surplus GSH and NAD^+. Endogenous formaldehyde is oxidized by ADH3 to formate which is either eliminated as CO_2 in the exhaled breath or used in the cellular macromolecular synthesis or excreted in urine. Similarly, the majority of inhaled formaldehyde is metabolized in the URT by conversion to formate. Further, part of it may be metabolized to CO_2 or utilized in the 1C pool. Since the available evidence does not show significant amounts of

exogenous formaldehyde being transported into blood, the subsequent clearance of any exogenous formaldehyde that does reach the blood should be similar to the handling of endogenous formaldehyde.

Excretion of formaldehyde

Inhalation exposure to formaldehyde has not been shown to cause significant changes to the tissue levels of formaldehyde in the nasal mucosa, the blood, or in the distal tissues. Thus, it is not expected that formaldehyde and formaldehyde metabolite content in excretion products would be altered by exposure. The data supporting this expectation are consistent in human and animal studies.

Formate levels have been detected in both unexposed as well as formaldehyde-exposed individuals. Gottschling et al. (1984) examined urinary formic acid levels of 35 veterinary medicine students working in an anatomy lab before exposure and within 2 hours following 1-, 2-, or 3-wk exposure to a mean formaldehyde concentration of <0.615 mg/m^3. The authors did not observe significant change in the pre- and postexposure levels of formic acid. Since co-exposure to methanol may also contribute to the metabolism and excretion of formate, the fact that no significant increase in urinary formate was seen even with that co-exposure further supports the conclusion that the formaldehyde exposure does not significantly increase formate excretion.

Heck et al. (1983) determined the relative contributions of various elimination pathways in F344 rats following inhalation exposure to 0.77 and 16.1 mg/m^3 of ^{14}C-formaldehyde. As shown in Table A-14, the percentages of radioactivity in various fractions appear to be similar between the two dose groups tested. Within 70 hours after a 6-hour formaldehyde exposure, nearly 40% of radioactivity from inhaled ^{14}C-formaldehyde appeared to be eliminated via expiration, probably as $^{14}CO_2$ (it should be recalled that nearly 100% of inhaled formaldehyde is taken up by the URT); and ≈17 and 5% of radioactivity was eliminated in the urine and feces, respectively. Nearly 40% of radioactivity remained in the carcass, which is presumably due to both covalent binding and metabolic incorporation. Thus, in one form or another, 40% of the ^{14}C from inhaled formaldehyde is not eliminated and is expected to persist in the tissue(s) for some time. Overall, the authors concluded that, in rats, the relative elimination pathways for the remaining 60% of the ^{14}C are independent of exposure concentration, and followed the pattern of elimination in the order of expired air > urine > feces.

Although not specifically demonstrated following exposure, assumptions based on the known distribution and metabolism of formaldehyde and its detoxification products allow for inferences to be drawn regarding how inhaled ^{14}C reaches these elimination points. Approximately one-third of inhaled formaldehyde is estimated to be removed in the URT mucus (Schlosser, 1999). It is expected that the majority of this formaldehyde would be removed from the URT via mucociliary clearance and excreted in urine in various forms. A large amount of inhaled formaldehyde penetrating the mucociliary layer of the URT is metabolized in the nasal cavity, giving

rise to formate, which can be excreted in urine. Part of this formate may also be further oxidized and eliminated in the exhaled breath as CO_2. Some formaldehyde is incorporated into the 1C pool.

Table A-14. Summary of excretion study following exposure to formaldehyde by inhalation in rats

Reference and species	Treatment and analysis	Observations		
Heck et al. (1983) Rats, Fischer Male, n=4 210 g	0.77 and 16.1 mg/m^3 HCHO for 6 hrs; rats sacrificed 70 hrs after removal from exposure chamber; tissues, urine, feces collected; exhaled $^{14}CO_2$ trapped in a solution of 5 M ethanolamine in 2-methoxyethanol and % radioactivity measured in LSC.	% Radioactivity (Mean ± SD) in various fractions		
		Source of radioactivity	Air borne CH_2O	
			0.77 mg/m^3	16.1 mg/m^3
		Expired air:	39.4 ± 1.45	41.9 ± 0.8
		Urine:	17.6 ± 1.2	17.3 ± 0.6
		Feces:	4.2 ± 1.5	5.3 ± 1.3
		Tissues[a] and carcasses:	38.9 ± 1.2	35.2 ± 0.5

[a]Nasal mucosa, trachea, esophagus, lung, kidney, liver, intestine, spleen, heart, plasma, erythrocytes, brain, testes.

Levels of endogenous formaldehyde in exhaled human breath

Given that inhaled formaldehyde is almost entirely captured in the URT and is thus unlikely to reach either the lower respiratory tract (LRT) or the systemic circulation to an appreciable extent following exposure, and given that formaldehyde inhalation does not appreciably change total formaldehyde levels in blood or any other tissue; it has been postulated that formaldehyde in exhaled breath (measured in mouth-only exhalations) is expected to predominantly represent a contribution from endogenous formaldehyde. However, it is important to understand the relative amount of formaldehyde that is produced by the body and released in expired breath versus the amount of formaldehyde in ambient air.

Table A-15 summarizes six studies that attempted to measure endogenous formaldehyde in exhaled breath. All studies performed prior to 2010 are limited by their analytical methods, which are subject to interference from other ions and isotopes that have the same *m/z* ratio (*m/z* = 31) as formaldehyde (e.g., methanol, ethanol, and nitric oxide). Also, it was not possible to differentiate between exogenous and endogenous formaldehyde in exhaled breath because the study subjects inhaled room air containing formaldehyde (≈11 µg/m^3 formaldehyde).

Table A-15. Measured levels of formaldehyde, methanol and ethanol in room air and exhaled breath

Study	Analytical Method	Sample	Formaldehyde c (m/z 31) µg/m^3	Methanol µg/m^3	Ethanol µg/m^3
Moser et al. (2005)[a]	PTR-MS DL: NR	Room air:	"Negligible"	"Negligible"	"Negligible"
		Exhaled breath:	5.24 (median)	198	NR

Study	Analytical Method	Sample	Formaldehyde c (m/z 31) µg/m³	Methanol µg/m³	Ethanol µg/m³
N = 344			1.49–89 (range)		
Kushch et al. (2008) *N* = 370	PTR-MS DL: NR	Room air:	NR	NR	NR
		Exhaled breath:	6.39 (median, nonsmokers) 5.53 (median, 81 smokers)	241 (median, nonsmokers)	NR
Cap et al. (2008) [b] *N* = 34	SIFT-MS DL: 3.68 µg/m³ or better	Room air:	11.79 ± 1.84	NR	NR
		Exhaled breath:	2.46 (mean) 1.23 (median) 0–14.74 (range) 0 and 3.68 in 2 smokers	365 (mean) 232 (median) 125–2,848 (range)	549 (mean) 101 (median) 33–12,604 (range)
Turner et al. (2008) *N* = 5	SIFT-MS DL: 6.14 µg/m³ or better	Room air:	ND	NR	NR
		Exhaled breath:	ND	617 (mean)	549 (mean)
Wang et al. (2008) *N* = 3	SIFT-MS DL: NR	Room air:	11.05 ± 3.68	54 ± 11	124 ± 63
		Exhaled breath:	6.51 (mean) 4.91–8.6 (range)	329 (mean)	185.46 (mean)
Riess et al. (2010) *N* = 8 (nonsmokers) N = 2 (smokers)	*Acac* method DL: <0.62 µg/m³ [d]	Charcoal filtered air:	0	NR	NR
		Exhaled breath:	<0.62 (nonsmokers), ND <0.62 (2 smokers), ND	NR	NR
	PTR-MS [e] DL: ≈0.62 µg/m³	Charcoal filtered air:	0	NR	NR
		Exhaled breath:	1.84 (mean; 0.86–2.82), nonsmokers; 1.23–2.82, 2 smokers	NA	NA

[a]Authors reported room air concentrations for 179 chemicals were "negligible." No smoker data were provided.
[b]Smoker data and formaldehyde ambient concentration provided by Dr. Španěl (personal communication).
[c]Values of formaldehyde in parts per billion (ppb) are converted as µg/m³ = ppb × 30 (m.w.)/24.45 or ppb × 1.23.
[d]The *acac* method's limit of detection is 0.062 µg formaldehyde/m³, but the authors calculated a detection limit of 0.62 µg/m³ due to a slight periodically fluctuating background noise signal.
[e]After subtraction for methanol and NO product ions.
Abbreviations: DL = Detection Limit; NR = Not Reported; ND = Not Detected; NA = Not Applicable; PTR-MS = Proton Transfer Reaction Mass Spectrometry; SIFT-MS −= Selected Ion Flow Tube Mass Spectrometry.

Riess et al. (2010), employed the acetyl acetone (*acac*) method[4] to measure formaldehyde. This method is superior to the PTR-MS method used in previous studies because it has a lower limit of detection, exhibits no interference from other exhaled chemicals, and possesses the ability to measure in dry or humid atmospheres. In addition, volunteers inhaled formaldehyde-free air. For comparison, Riess et al. (2010) used both the *acac* method and the PTR-MS method and observed

[4]The *acac* method entails the cyclization of 2, 4-pentanedione (*acac*), ammonium acetate, and formaldehyde to form dihydropyridine 3, 5-diacetyl-1, 4-dihydrolutidine (DDL), which fluoresces at 510 nm after excitation at 412 nm.

mean exhaled formaldehyde concentrations of 1.84 μg/m^3 in nonsmokers and 1.23–2.82 μg/m^3 in smokers by the PTR-MS method, but no detectable formaldehyde in any subjects (including smokers) by the formaldehyde-specific *acac* method (see Table A-15). A concentration of 5.13 μg/m^3 was detected by the *acac* method in a single smoker who was asked to smoke two cigarettes immediately before the measurement. This smoker's formaldehyde level declined below the level of detection within 30 min. Formaldehyde levels were 1.47 to 2.09 μg/m^3 in subjects asked to consume methanol-rich hard fruit liquor within 48 hours of the test (recall that methanol is metabolized by alcohol dehydrogenase to formaldehyde throughout the body). So, even when formaldehyde levels were intentionally elevated, very little endogenous formaldehyde was expelled in exhaled breath and these elevations were transient.

In summary, Riess et al. (2010), the only study to date which avoided the limitations of previous studies, demonstrated that if endogenous formaldehyde exists in exhaled breath, it is usually below their level of detection of <0.62 μg/m^3.

A.2.10. Conclusions Regarding the Toxicokinetics of Inhaled Formaldehyde Outside of the POE

In summary, the published data demonstrate that endogenous formaldehyde blood levels across species are approximately 0.1 mM and these levels do not change with exogenous formaldehyde exposure, arguing that inhaled formaldehyde is not absorbed into blood. One limitation of these studies is that these detection methods did not provide a clear distinction on the nature of formaldehyde (e.g., free, reversibly or irreversibly bound, measured as formate, or part of the 1C pool). Formaldehyde inhalation studies show metabolic incorporation, but not covalent binding (e.g., hm-DNA adducts and DPXs) in bone marrow of rats which conclusively show that exogenous formaldehyde is not transported to the distal tissues. Formaldehyde is likely to be metabolized in a similar way in distal tissues since enzymes required for metabolism are expressed in all the tissues. Endogenous levels of formaldehyde in exhaled breath analyzed by different research groups are often limited due to the lack of specificity in analytical methods and confounding by presence of formaldehyde in room air in these studies. Based on a recent improved method, endogenous formaldehyde concentrations in exhaled air have been detected to be lower than the study's detection limit of 0.62 μg/m^3 outside of exceptional circumstances (just after smoking two cigarettes or ingesting something with a high level of methanol).

A.2.11. Toxicokinetics Summary

Formaldehyde is an endogenous chemical produced intracellularly by enzymatic and nonenzymatic pathways during normal cellular metabolism and a relatively small fraction of free formaldehyde is produced from metabolism of xenobiotics. Studies in experimental animals using direct and indirect measurements and modeling studies in human subjects have clearly shown that a majority of inhaled formaldehyde is rapidly absorbed in the URT despite anatomical and physiological differences across species. Inhaled formaldehyde develops a concentration gradient

with an anterior to posterior distribution in the nasal cavity. High concentrations of formaldehyde are distributed to squamous, transitional, and respiratory epithelia; less formaldehyde uptake occurs in the olfactory epithelium, and very little or no formaldehyde reaches the lower respiratory tract, except possibly at very high exposure concentrations and/or during periods of high exertion with oronasal breathing. Studies in rats show that single exposure to high levels of formaldehyde or repeated exposure to varying concentrations does not appreciably change the tissue levels of formaldehyde over the endogenous levels in the nasal mucosa.

Inhaled formaldehyde entering the nasal cavity interacts with the mucociliary apparatus which is the first line of defense. The majority of formaldehyde is rapidly converted to methanediol (≈99.9%), with a minor fraction (≈0.1%) remaining as free formaldehyde in the nasal mucus. A rapid equilibrium is assumed such that the 99.9:0.1% ratio is maintained at all times. Methanediol penetrates the tissues while free formaldehyde reacts with the macromolecules. Uncertainties remain about the distribution of formaldehyde to underlying epithelium owing to the presence of endogenous formaldehyde, which is a component of normal cellular metabolism. Formaldehyde is metabolized to formate predominantly by ADH3 and by a minor pathway involving mitochondrial ALDH2. Formate can either enter the one-carbon pool leading to protein and nucleic acid synthesis or is further metabolized to CO_2 and eliminated in expired air or excreted in urine unchanged.

Formaldehyde can interact with macromolecules either noncovalently (GSH, THF) or covalently (DPX, DDX, hm-DNA monoadducts, protein adducts). In rats and monkeys, DPXs show dose-response in the nasal cavity where DPX distribution corresponds to tumor sites (rats) and cell proliferation (rats and monkeys), suggesting that DPX may be a good biomarker of exposure. Formaldehyde also induces a concentration-dependent increase in DNA monoadducts (e.g., N^2-hm-dG adducts) in the nasal passages of monkeys and rats which can be distinguished from endogenous adducts using improved analytical methods. Higher levels of endogenous N^2-hm-dG adducts are detectable than the exogenous monoadducts, except at the highest inhaled exposure concentrations.

The toxicokinetics of formaldehyde may be influenced by certain formaldehyde-induced effects, such as modifications to mucociliary clearance, reflex bradypnea (rodents only) and reduction in minute volume, and dynamic tissue remodeling (e.g., squamous metaplasia), which have the potential to modulate formaldehyde uptake and clearance. For example, inhaled formaldehyde induces mucostasis and ciliastasis in the rat nasal mucociliary apparatus extending from anterior to posterior regions of the nasal cavity depending on the concentration and duration of exposure. Thus, at least at higher concentrations (e.g., at low concentrations, formaldehyde does not clearly cause mucostasis), estimates of tissue formaldehyde levels may be more uncertain. Similarly, the differences observed in altered minute volumes in rats and mice during repeated inhalation exposure to formaldehyde may impact dosimetric adjustment if extrapolated to humans.

Endogenous blood formaldehyde levels average around 0.1 mM across different species and inhalation exposure to formaldehyde does not alter blood formaldehyde levels, suggesting that

inhaled formaldehyde is not significantly absorbed into blood. Formaldehyde-induced exogenous DNA monoadducts were detectable in nasal tissues but not in distal tissues of experimental animals exposed by inhalation. This argues against systemic transport of formaldehyde to distal tissues. Also, formaldehyde inhalation studies show metabolic incorporation, but not covalent binding in bone marrow of rats, further supporting the lack of transport of formaldehyde (as opposed to metabolites of formaldehyde) to the distal tissues.

Analysis of formaldehyde in exhaled breath can be confounded by interfering gases in the analytical techniques or can be confounded by the presence of formaldehyde in the room air. With improved techniques, endogenous formaldehyde concentrations in exhaled air have been detected to be usually lower than the detection limit of 0.62 μg/m^3. Overall, no evidence is available to indicate that inhaled formaldehyde is systemically transported.

A.2.12. Modeling Formaldehyde Flux to Respiratory Tract Tissue

Formaldehyde is highly reactive and water soluble, thus its absorption in the mucus layer and tissue lining of the respiratory tract is known to be significant. This absorption is highly regional and the absorption patterns differ substantially across species. This section first provides the motivation for developing detailed dosimetry models for the regional and species-specific absorption of formaldehyde. It then discusses the computation of inhaled formaldehyde transport in the upper (nose and mouth) and lower (lung and trachea) respiratory tract using fluid dynamic models, and evaluates the level of confidence in these predictions. Finally, a revised dosimetry model that incorporates estimates of endogenous formaldehyde is discussed.

Species differences in anatomy: consequences for gas transport and respiratory tract lesions

The regional dose of inhaled formaldehyde in the epithelial lining of the respiratory tract of a given species depends on the amount absorbed at the airway-tissue interface, water solubility, mucus-to-tissue phase diffusion, and chemical reactions, such as hydrolysis, protein binding, and metabolism, and on the amount of formaldehyde delivered by the inhaled air to the tissue lining. This is a function of the major airflow patterns, air-phase diffusion, and absorption at the airway-epithelial tissue interface. Formaldehyde-induced squamous cell carcinomas (SCC) and other lesions that occur in the rat and monkey nasal passages and in the monkey lower respiratory tract are seen to be localized, with the lesion distribution patterns also showing species-specificity. It has been argued that the main determinant of these patterns and their differences among species is regional dose (Bogdanffy et al., 1999; Monticello et al., 1996; Monticello and Morgan, 1994; Morgan et al., 1991).

The anatomy of the respiratory tract, in particular the upper part (see Figure A-10), and airflow patterns in this region (see Figure A-11) show large differences across species. Furthermore, because of the convoluted nature of the airways (see Figure A-10), the uptake of reactive and water-soluble gases such as formaldehyde in the upper respiratory tract (as seen in various simulations, Figure A-12) is highly nonhomogeneous over the nasal surface. Thus, as

shown in Figure A-12, the spatial distribution of formaldehyde flux also shows strong species dependence. These observations, when juxtaposed with the localized occurrence of lesions, suggest that regional dose may be important in reducing uncertainty when extrapolating risk-related dose across species. Kimbell et al. (1993), Kepler et al. (1998), and Subramaniam et al. (1998) developed anatomically realistic finite-element representations of the noses of F344 rats, rhesus monkeys, and humans, and used them in physical and computational models (Kimbell et al., 2001a; Kimbell et al., 2001b); see Figure A-10 and Figure A-11). This assessment uses dosimetry derived from these representations.

Formaldehyde dosimetry in the lower human respiratory tract (i.e., in the trachea and lung) may also be important to consider. The upper respiratory tract is generally a good scrubber of formaldehyde; as a result, there is less penetration into the lungs. However, the extent of this scrubbing varies among species. The rat upper respiratory tract is extremely efficient with only about 3% fractional penetration to the lower respiratory tract (Morgan et al., 1986a); however, penetration to the lung appears to be higher in the rhesus monkey (see Figure A-12). Accordingly, while frank effects were seen only in the upper respiratory tract in rodents, DPX lesions induced by exposure to 6 ppm formaldehyde were also present in the major bronchiolar region of the rhesus monkey (see Section 1) whose respiratory tract morphology is somewhat similar to the human (see Figure A-10 and Figure A-11). Another factor is that humans are oronasal breathers, with a significant fraction of the population breathing normally through the mouth (Niinimaa et al., 1981), while rats are obligate nose-only breathers. Oronasal breathing implies a much higher dose to the lower respiratory tract, particularly at higher activity profiles [see Figure A-13 and Figure A-14 and Niinimaa et al. (1981)]. For all these reasons, the cancer dose-response assessment based upon nasal tumors observed in the F344 rat includes an additional exercise involving the human lung, even though the lung is not identified as a target organ in the hazard assessment. The dose-response section evaluates the extent to which human risk estimates increase when formaldehyde dose to the lower human respiratory tract is also considered. The dosimetry modeling for this purpose uses an **idealized** single-path model of the lower respiratory tract developed by Overton et al. (2001) discussed later Appendix B.2.2.

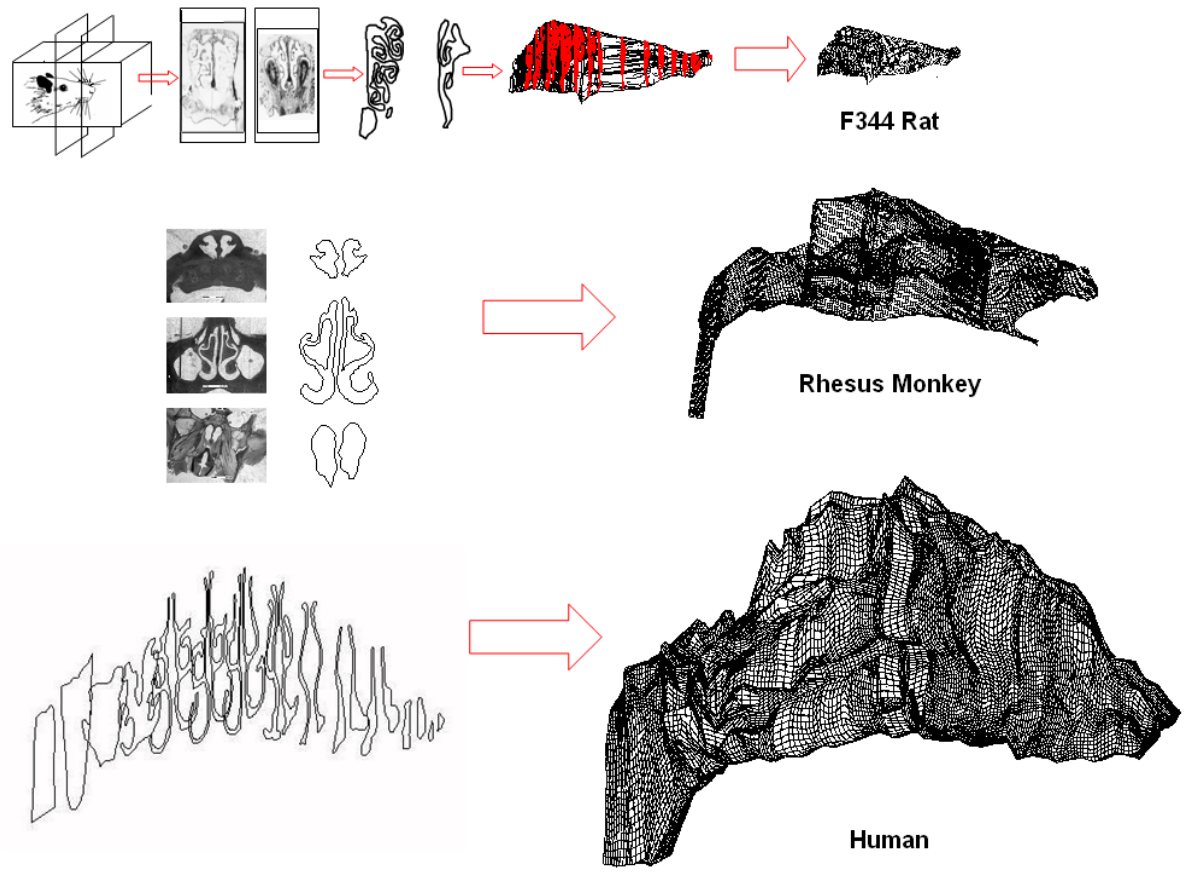

Figure A-10. Reconstructed nasal passages of F344 rat, rhesus monkey, and human.

Note: Nostril is to the right, and the nasopharynx is to the left. Right side shows the finite element mesh. Left-hand side shows tracings of airways obtained from cross sections of fixed heads (F344 rat and rhesus monkey) and magnetic resonance image sectional scans (humans). Aligned cross sections were connected to form a three-dimensional reconstruction and finite-element computational mesh. Source: Adapted from Kimbell et al. (2001b). Additional images provided courtesy of Dr. J.S. Kimbell, CIIT Hamner Institutes.

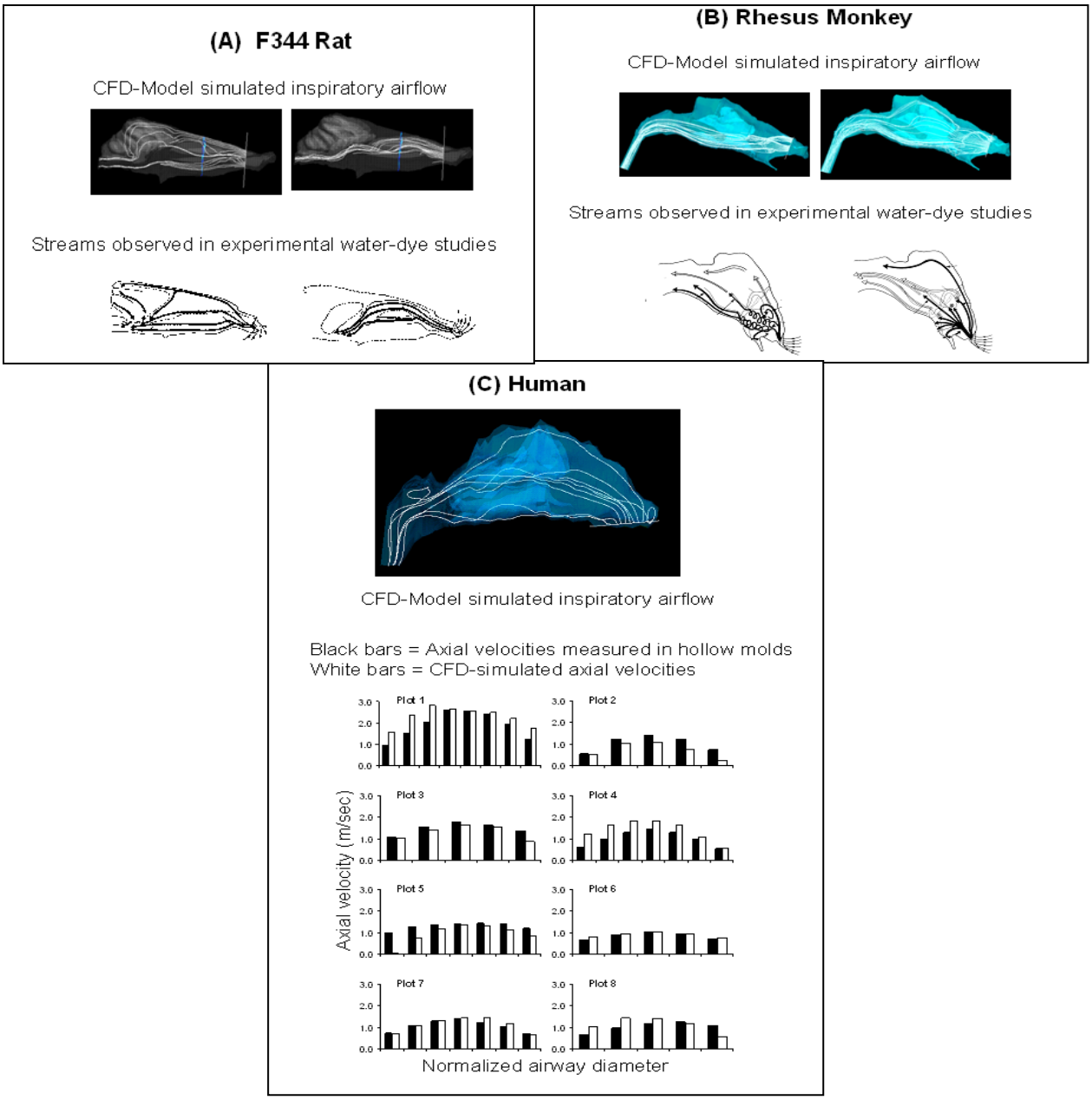

Figure A-11. Illustration of interspecies differences in airflow and verification of CFD simulations with water-dye studies.

Note: Panels A and B show the simulated airflow pattern versus water-dye streams observed experimentally in casts of the nasal passages of rats and monkeys, respectively. Panel C shows the simulated inspiration airflow pattern, and the histogram depicts the simulated axial velocities (white bars) versus experimental measurements made in hollow molds of the human nasal passages. Dye stream plots were compiled for the rat and monkey over the physiological range of inspiration flow rates. Modeled flow rates in humans were 15 L/min.

Source: Adapted from Kimbell et al. (2001b).

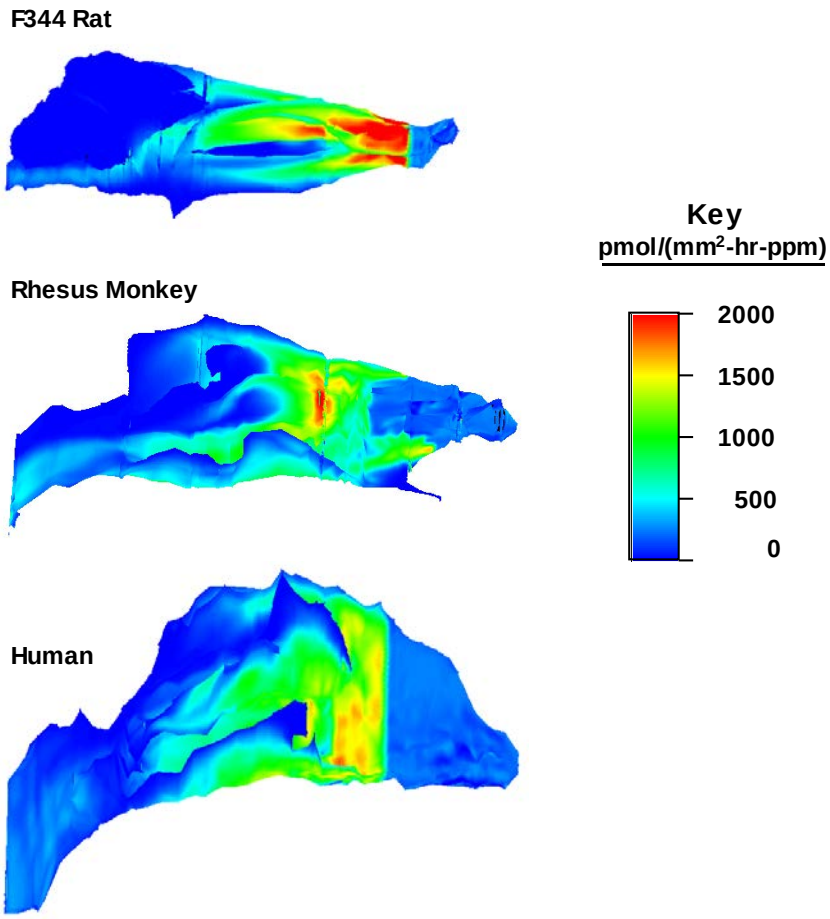

Figure A-12. Lateral view of nasal wall mass flux of inhaled formaldehyde simulated in the F344 rat, rhesus monkey, and human.

Note: This is a rendering of a three-dimensional surface. Nostrils are to the right. Simulations were exercised in each species at steady-state inspiration flow rates of 0.576 L/min in the rat, 4.8 L/min in the monkey, and 15 L/min in the human. Flux was contoured over the range from 0–2,000 pmol/(mm^2-hour-ppm) in each species.

Source: Kimbell et al. (2001b).

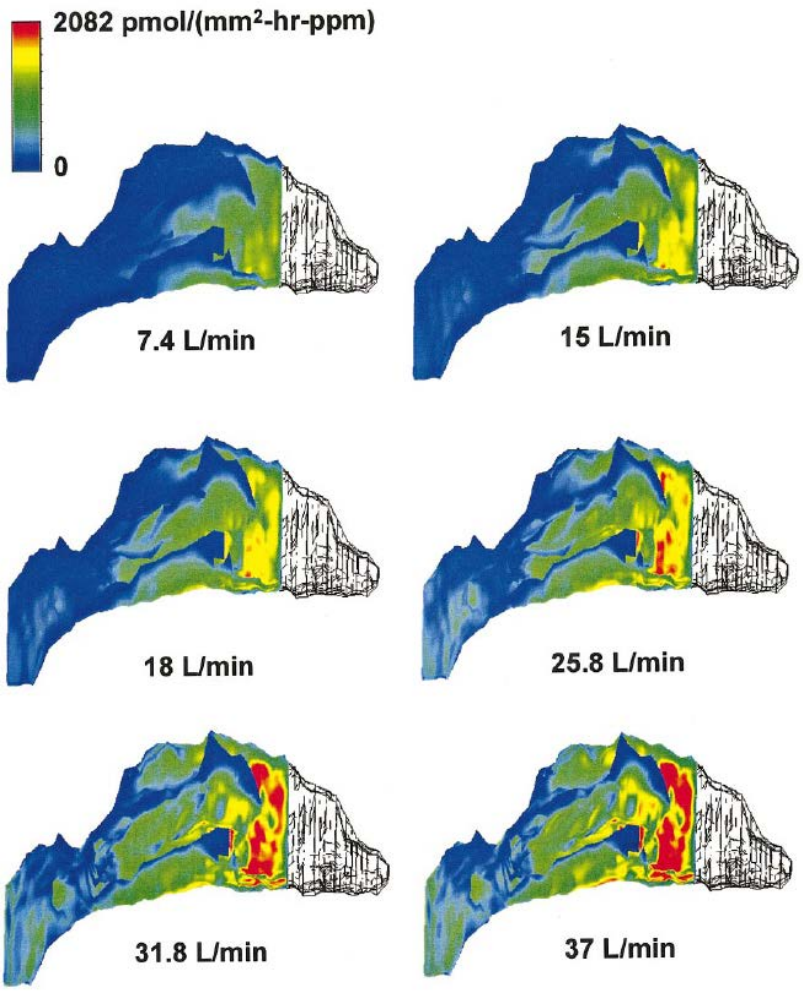

Figure A-13. Lateral view of nasal wall mass flux of inhaled formaldehyde simulated at various inspiratory flow rates in a human model.

Note: This is a rendering of a three-dimensional surface, showing the right lateral view. Uptake is shown for the nonsquamous portion of the epithelium. The front portion of the nose (vestibule) is lined with keratinized squamous epithelium and is expected to absorb relatively much less formaldehyde.

Source: Kimbell et al. (2001a).

Modeling formaldehyde uptake in nasal passages

Anatomical reconstruction and tissue types: The dose-response modeling results evaluated and used in this document are based on several published computational models for air flow and formaldehyde uptake in the nasal passages of a F344 rat[5], rhesus monkey, and human, and in the human lung (Kimbell et al., 2001b; Overton et al., 2001; Kepler et al., 1998; Subramaniam et al., 1998; Kimbell et al., 1993). The anatomical reconstructions for both computational and physical

[5] This strain of the rat is considered anatomically representative of its species and widely used experimentally, most notably in bioassays sponsored by the National Toxicology Program.

models were based on tracings of airways obtained from cross sections of fixed heads (F344 rat and rhesus monkey) and magnetic resonance image sectional scans (human).

Formaldehyde-induced nasal SCCs in rats are observed to arise only from respiratory or transitional epithelial cells in F344 rats and thought to be associated with the transformation of these cell-types to a squamous epithelial type due to exposure to formaldehyde (Morgan et al., 1986a). Therefore, the dosimetry calculations in Kimbell et al. (2001b) focused on predicting the wall mass flux of formaldehyde (rate at which mass of formaldehyde is transported to unit area of the nasal or lung lining prior to disposition within the body—mass/[area-time]) to regions lined by respiratory or transitional epithelium and excluding squamous epithelial cells. An additional distinction was made regarding these regions. Formaldehyde hydrolyses in water and reacts readily with a number of components of nasal mucus, and was therefore assumed to be absorbed at a higher rate by epithelial lining coated with mucus. The approximate locations of mucus-coated and nonmucus coated respiratory/transitional epithelial cells were mapped onto the reconstructed nasal geometry of the computer models. Types of nasal epithelium overlaid onto the geometry of the models were assumed to be similar in characteristics across all three species (rat, monkey, and human) except for thickness, surface area, location, and the extent of the nasal surface not coated by mucus. These characteristics were estimated from the literature or by direct measurements (Conolly et al., 2000; CIIT, 1999).

The fluid dynamics modeling in the respiratory tract comprises two steps: (1) model airflow through the airway lumen (solution of Navier-Stokes equations) and (2) using these solutions of the airflow field as input, model formaldehyde flux to the respiratory tract lining (solution of convective-diffusion equations). The local formaldehyde flux at the airway-to-epithelial tissue interface was assumed to be proportional to the air-phase formaldehyde concentration adjacent to the nasal lining. The proportionality constant is the mass transfer coefficient for the tissue phase, specified as boundary conditions on the solutions, and takes different values in the model depending on whether the tissue is coated with a mucus layer (k_m) or not (k_{nm}). Epithelium not coated with mucus was considered similar to epidermal tissue, and a value available from the literature for such tissue was used for k_{nm}. On the other hand, Kimbell et al. determined k_m empirically for the rat by fitting the overall nasal uptake predicted by the CFD model to the average experimental values obtained by Morgan et al. (1986a). The values of k_m and k_{nm} depend only on the solubility and diffusivity of the gas in the tissue, the thickness of tissue, and the reaction rate of the gas (Hanna et al., 2001). Tissue thickness varies across species, but because formaldehyde is highly reactive and soluble, the primary kinetic determinant of interspecies differences in the net mass transfer rate is likely the difference in air-phase resistance and not tissue thickness. Therefore, Kimbell et al. (2001b) assumed that values for the tissue phase mass transfer coefficients were the same for the human. EPA judges this assumption to be reasonable. The air-phase resistance (which is the inverse of the air-phase mass transfer coefficient) on the other hand would vary substantially between the rat and human on account of the substantial interspecies

variations in airway geometry and airflow discussed earlier. Details of the boundary conditions for air flow and mass transfer, are provided in Kimbell et al. (2001b; 2001; 1993) and Subramaniam et al. (1998).

For the rat, minute volumes were allometrically scaled to 0.288 L/minute for a 315 g rat (Mauderly, 1986), and simulations were carried out at the steady-state unidirectional inspiratory rate of 0.576 L/min. For the human, simulations were carried out at the steady-state unidirectional inspiratory rate of 15, 18, 50, and 100 L/min, corresponding to half of the values for the minute volumes associated with the activity patterns of sleeping, sitting, and light and heavy exercise, respectively (ICRP, 1994). Because formaldehyde is highly water soluble and reactive, Kimbell (2001b) assumed that uptake occurred only during inspiration. Thus, for each breath, flux into nasal passage walls (rate of mass transport in the direction perpendicular to the nasal wall per mm^2 of the wall surface) was assumed to be zero during exhalation, with no backpressure to uptake built up in the tissues. Overton et al. (2001) estimated the error due to this assumption to be small, roughly an underestimate of 3% in comparison to cyclic breathing. Inspiratory airflow was assumed to be constant in time (steady state). Subramaniam et al. (1998) considered this to be a reasonable assumption during resting breathing conditions based on a value of 0.02 obtained for the Strouhal number. Unsteady effects are insignificant when this number is much less than one. However, this assumption may not be reasonable for light and heavy exercise breathing scenarios.

Kimbell et al. (2001b) partitioned the nasal surface by flux to facilitate the use of local formaldehyde dose in dose-response modeling. Each of the resulting 20 "flux bins" was comprised of elements of the nasal surface that receive a particular interval of formaldehyde flux per ppm of exposure concentration (Kimbell et al., 2001b). These elements were not necessarily contiguous. The spatial coordinates of elements comprising a particular flux bin were fixed for all exposure concentrations, with formaldehyde flux (pmol/(mm^2-hour) in a bin scaling linearly with exposure concentration (ppm), and therefore often expressed in terms of flux per ppm, that is, pmol/(mm^2-hour-ppm).

Mass flux was estimated for the rat, monkey, and human over the entire nasal surface and over the portion of the nasal surface that was lined by nonsquamous epithelium (lateral wall mass flux shown in Figure 12). Formaldehyde flux was also estimated for the rat and monkey over the areas where cell proliferation measurements were made (Monticello et al., 1991; Monticello et al., 1989) and over the anterior portion of the human nasal passages that is lined by nonsquamous epithelium. Maximum flux estimates for the entire upper respiratory tract were located in the mucus-coated squamous epithelium on the dorsal aspect of the dorsal medial meatus near the boundary between nonmucus and mucus-coated squamous epithelium in the rat, at the anterior or rostral margin of the middle turbinate in the monkey, and in the nonsquamous epithelium on the proximal portion of the mid-septum near the boundary between squamous and nonsquamous epithelium in the human (see see Kimbell et al., 2001a, for tabulations of comparative estimates of formaldehyde flux across the species, for tabulations of comparative estimates of formaldehyde flux

across the species). The rat-to-monkey ratio of the highest site-specific fluxes in the two species was 0.98. In the rat, the incidence of formaldehyde-induced SCCs in chronically exposed animals was high in the anterior lateral meatus [ALM, Monticello et al. (1996)]. Flux (per ppm of inhaled concentration) at this site in the rat was similar to that predicted near the anterior or proximal aspect of the inferior turbinate and adjacent lateral walls and septum in the human, with a rat-to-human ratio of 0.84.

Formaldehyde Uptake in The Lower Respiratory Tract

Unlike the nasal passages, the human lower respiratory tract lends itself to a more simplified or idealized rendering. The one-dimensional (known as a "single-path" model) rendering of the human lung anatomy by Weibel (1963), which captures the geometry of the airways in an average or homogeneous sense for a given lung depth, is generally considered adequate unless the fluid dynamics at locations of airway bifurcations need to be explicitly modeled. Such an idealization of lung geometry has been successfully used in various models for the dosimetry of ozone and particulate and fibrous matter.[6] The single-path model was used to calculate formaldehyde uptake in the human lower respiratory tract (Overton et al., 2001; CIIT, 1999). These authors applied a one-dimensional equation of mass transport to each generation of an adult human symmetric, bifurcating Weibel-type respiratory tract anatomical model. In order to achieve consistency with the inhaled output from the CFD model of the upper respiratory tract in Subramaniam (1998), Overton et al. (2001) augmented their model with an idealized upper respiratory tract and constrained their one-dimensional version of the nasal passages to have the same inspiratory air-flow rate and uptake during inspiration as the CFD simulations.

[6] Such idealized representations are likely to be inappropriate for considering susceptible individuals, such as those with chronic obstructive pulmonary disease.

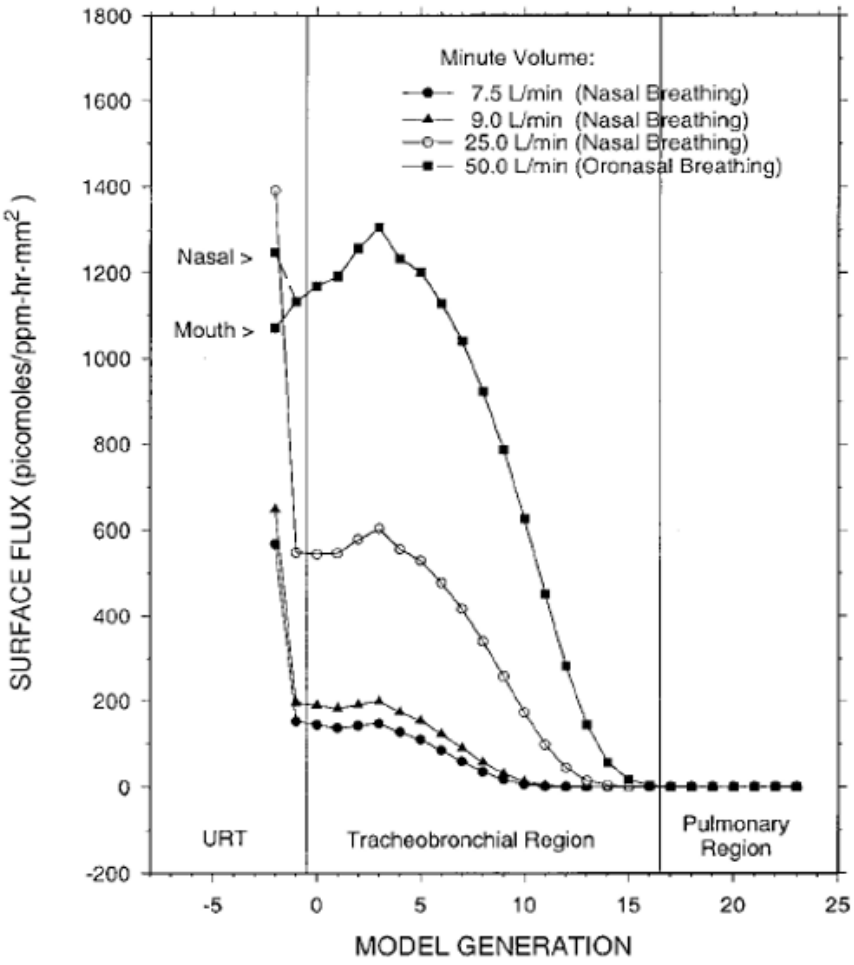

Figure A-14. Single-path model simulations of surface flux per ppm of formaldehyde exposure concentration in an adult male human.

Source: Overton et al. (2001).

The primary predictions of the model were: more than 95% of the inhaled formaldehyde is retained; formaldehyde flux in the lower respiratory tract increases for several lung airway generations relative to flux in posterior-most segment of the nose; with further increase in lung depth, formaldehyde flux decreases rapidly resulting in almost zero flux to the alveolar sacs. Overton et al. (2001) also modeled uptake at high inspiratory rates. At a minute volume of 50 L/minute[7] formaldehyde flux in the mouth cavity is comparable (but a bit less) to that occurring in the nasal passages (see Figure A-14).[8]

[7] Note: the oronasal switch occurs at about 35 L/min (Niinimaa et al., 1981).

[8] Mouth breathers form a large segment of the population. Furthermore, at concentrations of formaldehyde where either odor or sensory irritation becomes a significant factor, humans are likely to switch to mouth breathing even at resting inspiration. Overton et al. (2001) did not model uptake in the oral cavity at minute volumes less than 50

Level of confidence in formaldehyde uptake simulations

As mentioned earlier, the computational fluid dynamics simulations involved two steps, and the confidence in each step is addressed separately below.

Confidence in predicted airflow profiles

To verify the CFD simulations of nasal airflow profiles, the authors constructed physical models from the finite-element reconstructions used in the computational models. The simulated streamlines of steady-state inspiration airflow predicted by the CFD model agreed reasonably well with experimentally observed patterns of water-dye streams made in casts of the nasal passages for the rat and monkey as shown in panels A and B in Figure A-11. The airflow velocity predicted by CFD model simulations of the human also agreed well with measurements taken in hollow molds of the human nasal passages (see panel C, Figure A-11) (Kepler et al., 1998; Subramaniam et al., 1998; Kimbell et al., 1997b; Kimbell et al., 1993). However, the accuracy and relevance of these comparisons are limited. Because the airflow profiles were verified by only a simple video analysis of dye streak lines observed in the physical molds this method can be considered reasonable for only the major airflow streams. For the human, axial airflow velocities were also measured experimentally in a physical cast, and these compared well with CFD simulations (see panel C in Figure A-11). However, the physical model used for the velocity measurements corresponds to that of a different individual than the one for which the CFD simulations were carried out.

Another verification comes from measuring pressure gradients across the nasal cavity. Plots of pressure drop versus volumetric airflow rate predicted by the CFD simulations compared well with measurements made in rats in vivo (Gerde et al., 1991) and in acrylic casts of the rat nasal airways (Cheng et al., 1990) as shown in Figure A-15. This latter comparison remains qualitative due to differences among the simulation and experiments as to where the outlet pressure was measured and because no tubing attachments or other experimental apparatus were included in the simulation geometry. The simulated pressure drop values were somewhat lower, possibly due to these differences.

Kimbell et al. (2001a) examined the extent to which their results were subject to errors in mass balance and applied ad-hoc corrections to compensate for these errors. Because airflow and uptake were simulated separately, they each contributed separately to the mass balance error; however, the error component due to airflow was minimal (< 0.4%). The percent overall uptake of formaldehyde was defined as 100% × (mass entering nostril − mass exiting outlet)/(mass entering nostril), and its mass balance error was calculated as 100% × (mass entering nostril − mass

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L/min. However, since 0.55 of the inspired fraction is through the mouth for the normal nasal breathing population (Niinimaa et al., 1981) at an inspiratory rate of 50 L/min, we can make an indirect inference from their result at this heavy breathing rate that average flux across the human mouth lining would be comparable to the average flux across the nasal lining computed in Kimbell et al. (2001b; 2001) for mouth breathing conditions at resting or light exercise inspiratory rates.

absorbed by airway walls – mass exiting outlet)/(mass entering nostril). For the rat, monkey, and human the mass balance errors associated with simulated formaldehyde uptake from air into tissue were less than 14% at resting minute volumes, and therefore, not a major concern, but these errors increased to 27% at the highest human inspiratory rate corresponding to exercise conditions. Kimbell (2001a) corrected for these errors by evenly distributing the lost mass over the entire nasal surface in their simulation results.

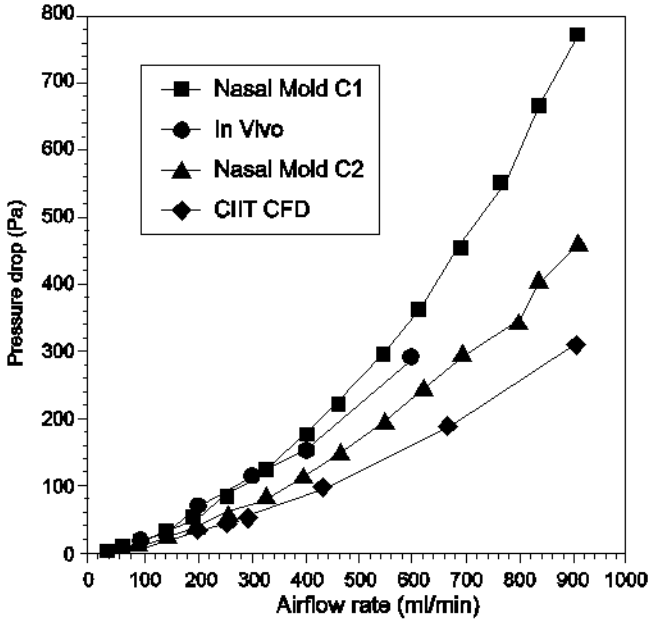


Figure A-15. Pressure drop versus volumetric airflow rate predicted by the CIIT CFD model compared with pressure drop measurements made in two hollow molds (C1 and C2) of the rat nasal passage (Cheng et al., 1990) or in rats in vivo (Gerde et al., 1991).

Source: Kimbell et al. (1997b).

Confidence in modeled flux estimates

Unlike the verification of the airflow simulations, it was not possible to evaluate the regional formaldehyde flux calculations directly; however, there are several indirect qualitative and quantitative lines of evidence that provide general confidence in the flux profiles predicted by Kimbell et al. (2001b; 2001) for the F344 rat nasal passages when the flux is averaged over gross regions of the nasal lining. This evidence is listed below.

In Kimbell (2001b), the tissue-phase mass-transfer boundary conditions were set by fitting overall (whole nose) formaldehyde uptake at various exposure concentrations to the experimental data in Morgan et al. (1986a). Since this was the only data set available, it was not possible to independently verify the model results for overall uptake. However, results from earlier work by Kimbell et al. (1993) are informative for this purpose because in this case the model was not

calibrated by fitting model predictions to experimental data; instead, this model assumed an infinite sink for absorption at the nasal lining on account of the highly reactive and soluble nature of formaldehyde. Kimbell et al. (1993) predict 99% uptake of inhaled formaldehyde in the rat nose, which is slightly above the upper end of the range of 91–98% observed by Morgan et al. (1986a). The utility of those simulations is however limited because the posterior portion of the nose was not included in the model, and the assumption of infinitely absorbing nasal walls makes the boundary condition less realistic than that used in Kimbell et al. (2001b). Calculations based upon Kimbell et al. (1993) are compared with various experimental observations below.

Morgan et al. (1991) showed general qualitative correspondence between the main routes of flow and lesion distribution induced by formaldehyde in the rat nose and hypothesized that the localized nature of the lesions must be related to the regional uptake of formaldehyde. This was borne out by Kimbell et al. (1993) who described similarities in patterns of computed regional mass flux and lesion distribution due to formaldehyde. These authors reported on correlations in patterns in the coronal section immediately posterior to the vestibular region (as discussed earlier, the vestibular region is protected by keratinized epithelium and is therefore not likely to significantly absorb formaldehyde); simulated flux levels over regions where lesions were seen, such as the medial aspect of the maxilloturbinate and the adjacent septum, were an order of magnitude higher than over other regions where lesions were not seen, such as the nasoturbinate.[9]

A reasonable level of confidence in flux predictions by Kimbell et al. (1993) is also attained indirectly by comparing experimental data on formaldehyde-DPX concentration in the F344 rat with modeled results in Cohen Hubal et al. (1997); these authors used flux estimates generated by the CFD model in Kimbell et al. (1993) in a physiologically based pharmacokinetic (PBPK) model for formaldehyde-DPX concentration in the F344 rat. This hybrid CFD-PBPK model was calibrated by optimizing model predictions of DPX concentrations against DPX collected over the entire nose in separate experiments by Casanova et al. (1991; 1989) on F344 rat noses exposed to formaldehyde at 0.3, 0.7, 2.0, 6.0, and 10 ppm. The nasal regions were then separated into two categories depending upon whether tumor incidence was high or low in a region, and model predictions of DPX concentrations were compared with the experimental data considered only from the high-tumor region, including additional DPX data from the high-tumor region at 15-ppm exposure concentration which had not been used in model calibration. The predictions are seen to compare well with experimental values (see Figure A-16). Such a comparison is not available for the simulation of uptake patterns in the human.

[9]This 1993 CFD model differed somewhat from the subsequent model by Kimbell et al. (2001b) used in this assessment. In the 1993 model, the limiting mass-transfer resistance for the gas was assumed to be in the air phase; that is, the concentration of formaldehyde was set to zero at the airway lining. Furthermore, this same boundary condition was used on the nasal vestibule as well, while in the more recent model, the vestibule was considered to be nonabsorbing. Unfortunately, Kimbell et al. (2001b) did not report on correspondences between flux patterns and lesion distribution.

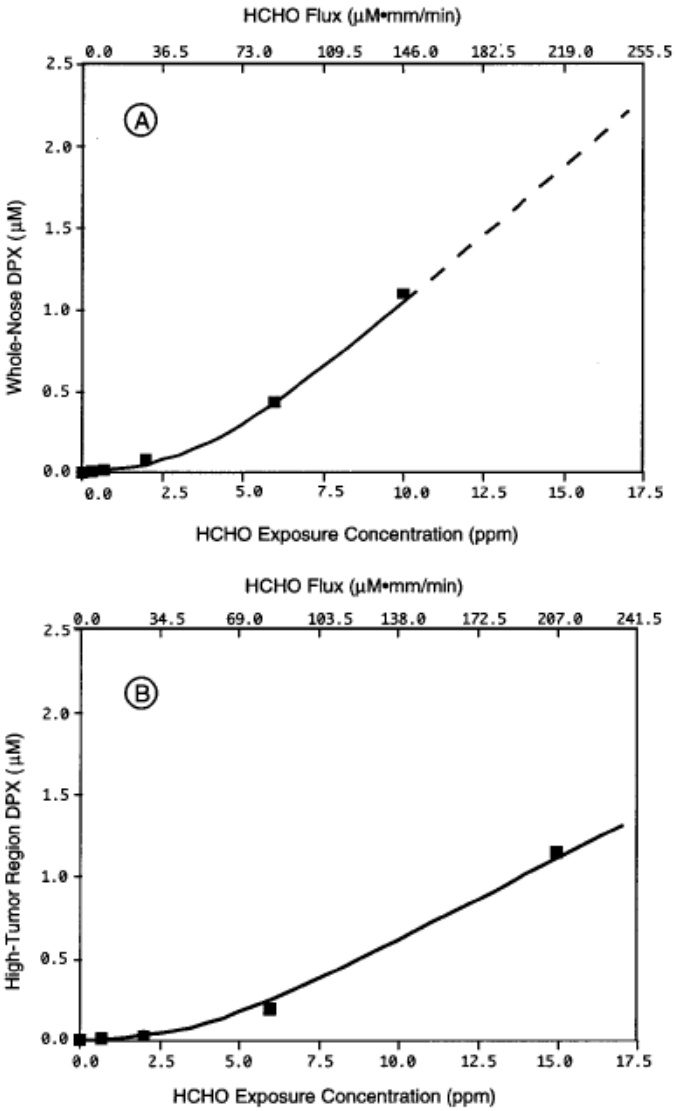


Figure A-16. Formaldehyde-DPX dosimetry in the F344 rat.

Panel A: calibration of the PBPK model using data from high and low tumor incidence sites. Panel B: model prediction compared against data from high tumor incidence site. Dashed line in panel A shows the extrapolation outside the range of the calibrated data.

Source: Cohen Hubal et al. (1997).

Effect of reflex bradypnea on dosimetry

A source of uncertainty in the modeled human flux estimates arises because the value of the tissue-phase mass-transfer coefficient used as a boundary condition in human simulations is the same as that obtained from calibration of the rat model. As explained earlier, qualitatively this appears reasonable; however, EPA is unable to quantitatively evaluate the impact of this uncertainty.

The CFD simulations do not model reflex bradypnea, a protective reflex observed in rodents. As discussed at length in Section A-3, it is reasonable to expect a range of 25% (Chang et al., 1983) to 45% (Barrow et al., 1983) decrease in minute volume in F344 rats at the exposure concentration of 15 ppm. Explicit omission of this effect in the modeling is, however, not likely to be a source of major uncertainty in the modeled results for uptake of formaldehyde in the rat nose for the following reason: the CFD model for the F344 rat was calibrated to fit the overall experimental result for formaldehyde uptake in the F344 rat at 15 ppm exposure concentration by adjusting the mass transfer coefficient used as boundary condition on the absorbing portion of the nasal lining. Thus, any reflex bradypnea occurring in those experimental animals is implicitly factored into the value used for the boundary condition. Nonetheless, some error in the localized distribution of uptake patterns may be expected, even if the overall uptake is reproduced correctly.

Modeling Interindividual Variability in the Nasal Dosimetry of Reactive and Soluble Gases

Garcia et al. (2009) used computational fluid dynamics to study human variability in the nasal dosimetry of reactive, water-soluble gases in 5 adults and 2 children, aged 7 and 8 years. The authors considered two model categories of gases, corresponding to maximal and moderate absorption at the nasal lining. We focus here only on the "maximal uptake" simulations in Garcia et al. (2009); note that this term for the simulations does not correspond to regions of maximum flux but rather characterizes the gas category. In this case, the gas was considered so highly reactive and soluble that it was reasonable to assume an infinitely fast reaction of the absorbed gas with compounds in the airway lining. Although such a gas could be reasonably considered as a proxy for formaldehyde, these results cannot be fully utilized to inform quantitative estimates of formaldehyde dosimetry (and does not appear to have been the intent of the authors either). This is because the same boundary condition corresponding to maximal uptake was applied on the vestibular lining of the nose as well as on the respiratory and transitional epithelial lining on the rest of the nose. This is not appropriate for formaldehyde as the lining on the nasal vestibule is made of keratinized epithelium which is considerably less absorbing than the rest of the nose (Kimbell et al., 2001a).

Garcia et al. (2009) concluded that overall uptake efficiency, and average and maximum flux levels over the entire nasal lining did not vary substantially between adults (1.6-fold difference in average flux and much less in maximum flux), and the mean values of these quantities were comparable between adults and children. These results are also in agreement with conclusions reached by Ginsberg et al. (2005) that overall extrathoracic absorption of highly and moderately reactive and soluble gases [corresponding to Category 1 and 2 reactive gases as per the scheme in U.S. EPA (1994)] is similar in adults and children. On the other hand Garcia et al. (2009) state that their models predicted significant interhuman variability in flux levels at specific points on the nasal wall; Figure 6A of their paper (reproduced here as Figure A-17) indicates a 3- to 5-fold difference among the individuals in the study when flux was plotted as a function of distance from the nostrils normalized by the length of the septum. This observation needs to be accompanied by

a caveat: because similar fluxes may correspond to different regions in individuals, it is possible that this spread in values overestimates the actual variability in local flux in these individuals.

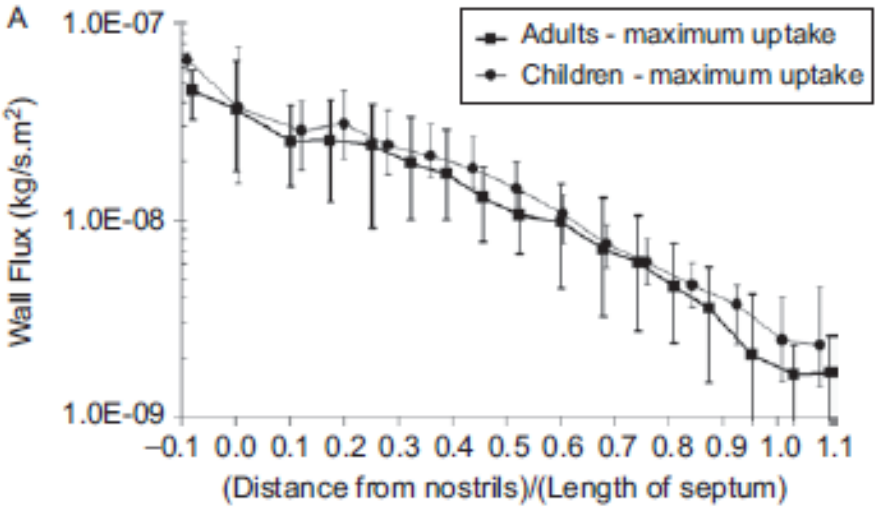


Figure A-17. Flux of highly reactive gas across nasal lining as a function of normalized distance from nostril for 5 adults and 2 children.

While the sample size in this study is too small to consider the results representative of the population as a whole, various comparisons with the characteristics of other study populations add to the strength of this study; for example, the surface area to volume ratio among the five adults ranged from 0.87 to 1.12 mm^{-1} which compared well with a result of 1.05 ± 0.23 obtained from measurements in 40 adult Caucasians (Yokley, 2009), and the surface area ranged from 16,683 to 23,219 cm^2 which compared well with a result of 18,300 ± 2,200 cm^2 obtained from measurements in 45 adults (Guilmette et al., 1997). It is useful to note here that the nasal anatomy reconstructed for modeling the dosimetry of formaldehyde in the human nose in Kimbell et al. (2001b; 2001) and discussed earlier was that of one of the individuals in the Garcia et al. (2009) study.

Models Estimating the Effects of Endogenous Formaldehyde on Dosimetry Predictions in Nasal Tissues

Schroeter et al. (2014) developed a hybrid toxicokinetic fluid dynamic model for predicting the uptake of inhaled formaldehyde that incorporates the production of endogenous formaldehyde in nasal tissue, and estimated a net decrease in uptake of inhaled formaldehyde at the lowest exposure concentrations based on modeling assumptions regarding the intracellular concentration of endogenous formaldehyde. More specifically, due to endogenous formaldehyde production, the model of Schroeter et al. (2014) predicts a net desorption of formaldehyde at zero exposure and that an external exposure between 1.23 μg/m^3 and 12.3 μg/m^3 (0.001 and 0.01 ppm) is required before there is sufficient air concentration to cause a net uptake of formaldehyde. However, any

external exposure is predicted to cause some, albeit very small, increase in the tissue concentration, since a nonzero air concentration reduces the net efflux of endogenous formaldehyde. While the analysis of Schroeter et al. (2014) represents an important first step towards incorporating the presence of endogenous formaldehyde into models estimating the flux (or uptake) of inhaled formaldehyde, several uncertainties in the underlying assumptions have yet to be addressed:

- Endogenous formaldehyde levels were calculated based on blood concentrations. But Heck et al. (1982) measured 12.6 μg/g total formaldehyde in rat nasal tissues and only 2.24 μg/g in rat blood (Heck et al., 1985).

- Based on DNA-adduct measurements, it appears that the majority of formaldehyde is bound to GSH in a manner that reduces its interaction with DNA and, presumably, other key macromolecules (see Section A.1.1.3.3.3). The extent of GSH-binding could significantly reduce diffusion across the epithelial cell membrane (i.e., between blood and nasal tissue), in which case blood concentrations may not correlate well with tissue concentrations.

- Since nasal tissue levels of formaldehyde are higher than blood levels, it is likely that these levels are produced by endogenous metabolism in situ, rather than entering the mucosa via diffusion from a "blood" layer at a specific depth from the mucosa-air surface, the latter being the assumption used by Schroeter et al. (2014).

- The tissue levels of formaldehyde predicted by the model of Schroeter et al. (2014) appear to be orders of magnitude in excess of the levels that would be consistent with the observed DPX levels (Heck et al., 1983) and formaldehyde-DNA binding rate (Heck and Keller, 1988).

- While Schroeter et al. (2014) did not report exhaled breath levels, their results indicate that uptake will exactly balance desorption in humans at about 1.23 μg/m^3 (0.001 ppm or 1 ppb), from which one might assume this is the level their model would predict in exhaled breath. In the study of Riess et al. (2010), exhaled breath levels for nonsmokers were found to be below a detection limit of 0.62 μg/m^3, which corresponds to 0.5 ppb at 20°C. While this is within a factor of two, an acceptable level of error for such an extrapolation, it is a further indication that the assumed level of free endogenous formaldehyde in the Schroeter et al. (2014) model is too high.

Despite these limitations, the efforts by Schroeter et al. (2014) highlight the fact that at sufficiently low levels of exogenous formaldehyde, the contribution of endogenous formaldehyde could become significant; accounting for this contribution would address a critical uncertainty for interpreting the uptake of inhaled formaldehyde. Additional studies addressing the potential contribution of endogenous formaldehyde are warranted. As discussed in the Toxicological Review (see Section 2.2.1), the unit risk estimate for nasal cancers based on rat studies are not appreciably altered if calculated using the revised formaldehyde estimates from Schroeter et al. (2014).

Campbell et al. (2020) modified the original model by Andersen et al. (2010) using exogenous and endogenous formaldehyde adduct data from Leng et al. (2019) (28-day study of 6 hrs/day exposures), Yu et al. (2015b) (28-day study of 6 hrs/day exposures), and Lu et al. (2011; 2010a) (a single 6-hour exposure). The following major changes were made to the original model:

a) The model simulates observed data for formaldehyde-induced DNA mono-adducts (N2-hydroxymethyl-dG). The previous models simulated formaldehyde-induced DNA-protein cross-links (DPX).
b) A zero-order term (VMMUC) was used to account for tissue clearance of inhaled formaldehyde. This is a restriction on uptake from the air phase to the tissue compartment.
c) The rate of production of endogenous formaldehyde (Kp) was increased to nearly double the original rate set by Andersen et al. (2010). The maximum rate of formaldehyde oxidase metabolism (Vmax) was increased by over a factor of 10.

There are some notable observations from the data used in the modeling. Leng et al. (2019) showed no exogenous formaldehyde-induced DNA adducts in the nose at concentrations up to 0.3 ppm and no increase in endogenous formaldehyde-induced DNA adducts up to 0.3 ppm. Lu et al. (2011; 2010a) observed an increase in exogenous formaldehyde adducts in rat nasal tissue starting at 0.7 ppm but no increase in endogenous adducts between 0.7 ppm–15 ppm (although there does appear to be a perturbation in the mean and variance of endogenous adducts in this range). The data at and above 0.7 ppm was used to re-optimize the cellular metabolic parameters. The data up to 0.3 ppm by Leng et al. (2019) (which did not observe increased adducts) was used to visually optimize the parameter defining the lower limit on uptake (VMMUC). Because of the abrupt change in observed adduct levels between 0.3 ppm and 0.7 ppm there is model uncertainty within that concentration range and below the limit of detection.

Key results from this work add to our characterization of uncertainties related to endogenous formaldehyde levels and formaldehyde dose-response at low exposures. First, the model estimated a non-zero value for VMMUC, indicating that the inhalation rate must exceed the tissue clearance rate for formaldehyde to be absorbed by the tissue. The model was calibrated with the restriction that formaldehyde absorption in the nose occurs only at exposure concentrations above 0.3 ppm in the rat. Secondly, Campbell et al. (2020) assessed steady-state concentration of free endogenous formaldehyde to be 20 times lower than the value determined experimentally by Heck et al. (1982) and 15 times lower than assessed by Andersen et al. (2010). In Campbell et al. (2020), the estimate for free endogenous levels decreased from 0.31 mM to 0.020 mM and the basal concentration of endogenous formaldehyde bound to sulfhydryl increased from 0.057 to 0.12mM (2 times higher). Campbell et al. (2020) attributed this discrepancy to the potential for the Heck et al. (1982) measurement methodology to overestimate tissue formaldehyde levels.

The original model (Andersen et al., 2010) did not adequately fit these new data, and Campbell et al. (2020) justified changes to the Andersen et al. (2010) model parameters for cellular metabolism on the grounds that data from Heck et al. (1982) are biased due to the method used to measure tissue formaldehyde. However, it is possible that the cause of this model/data discrepancy is inadequate model structure rather than a bias in the original data. As a result, there is inherent model uncertainty in the revised model for cellular metabolism.

Extrapolation of results in Campbell et al. (2020) to humans is not possible because the data and the model are specific to rats.

A.3. REFLEX BRADYPNEA

Reflex bradypnea (RB) is a protective reflex that allows laboratory rodents to minimize their exposure to upper respiratory tract (URT) irritants such as aldehydes, ammonia, isocyanates, and pyrethroids (Gordon et al., 2008). This reflex is initiated by stimulation of trigeminal nerve endings in the mucosa of the URT and the eyes. It is associated with the chemosensitive part of the nociceptive system—the common chemical sense that detects noxious airborne exposures (Nielsen, 1991).

The signs of reflex bradypnea: RB is manifest by immediate decreases in the metabolic rate, CO_2 production, and demand for oxygen. This is followed by rapid decreases in body temperature (i.e., hypothermia; as much as 11°C in rats and 14°C in mice; Figure A-18), activity, heart rate, blood pressure, respiratory rate (breaths/minute; Figure A-19), and minute volume (see Figure A-20). RB also results in decreased blood pO_2 and pCO_2 and increased blood pH (see Figure A-21) (Pauluhn, 2018; OECD, 2009; Gordon et al., 2008; Pauluhn, 2008; Chang and Barrow, 1984; Jaeger and Gearhart, 1982). Thus, the physiological effects and signs of RB may be misinterpreted as, for example, chemical-induced behavioral or developmental effects.

RB is regulated by a complex feedback response (Yokley, 2012). Gordon et al. (2008) demonstrated that the extent of RB depends on the concentration of the irritant (see Figure A-18). For example, after several hours of exposure to an isocyanate, mice exhibited concentration-dependent changes with those in the high concentration group presenting a mean body temperature of 23°C and approximately 90% decreases in respiratory rate and minute volume.

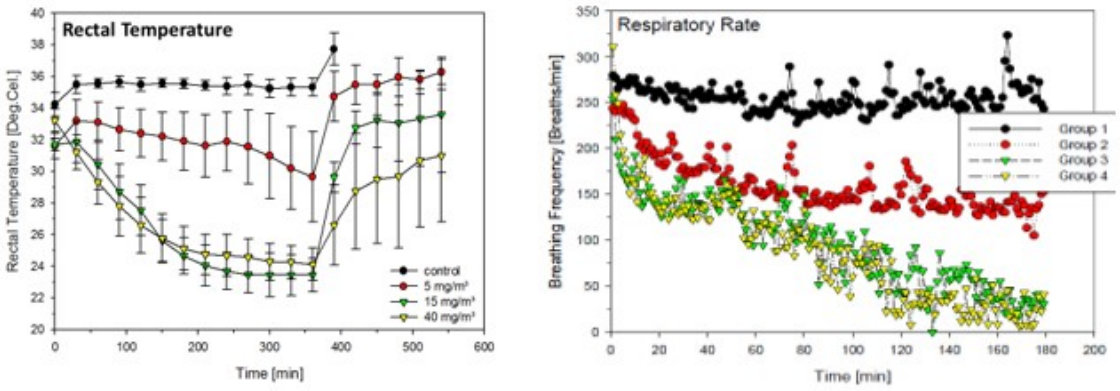


Figure A-18. Signs of Reflex Bradypnea. Left Panel: Concentration-related hypothermia in mice exposed to an isocyanate for 360 minutes. Note the gradual recovery in body temperature after exposure ceased. Right panel: Concentration-related decreases in respiratory rate in mice exposed to an isocyanate. Note the

correlation between the curves for rectal temperature and respiratory rate over the course of 180 minutes.

Source: Gordon et al. (2008).

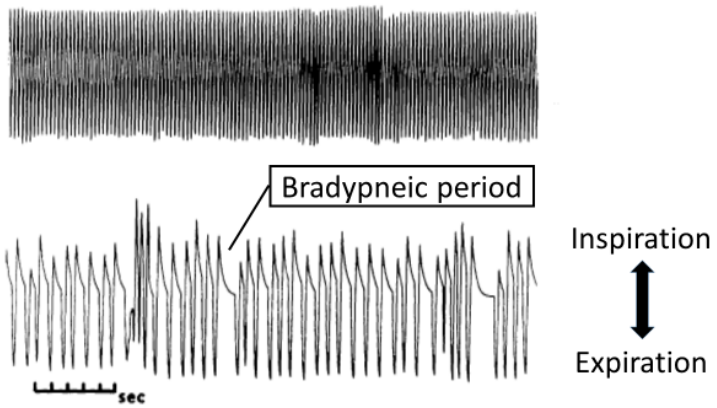


Figure A-19. An oscillograph that compares the respiratory cycle for mice exposed to an URT irritant (lower tracing) to an air control group (upper tracing). The exposed animals have a characteristic pause before exhaling—a bradypneic period—which results in a net decrease in the respiratory rate (breaths/minute). Because the exposed group has a slightly greater tidal volume (height of the tracings) but a much lower respiratory rate, the net result is a lower minute volume and reduced exposure to the irritant.

Source: Kane and Alarie (1977).

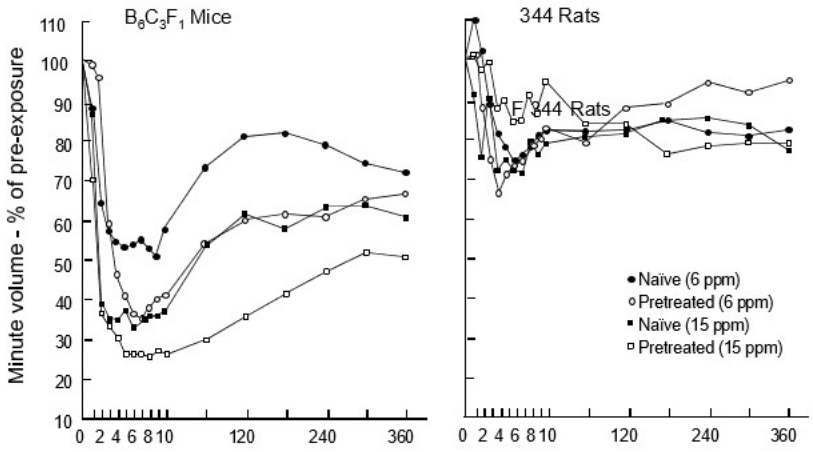


Figure A-20. Formaldehyde effects on minute volume in naïve and formaldehyde-pretreated male B6C3F1 mice and F344 rats. Pretreated animals were exposed to 6.9 or 17.6 mg/m^3 formaldehyde 6 hrs/d for 4 d. Note that the mice had a greater response than the rats, and the pretreated animals had a greater response than the naïve animals.

Source: Redrawn from Chang et al. (1983).

Figure A-20 demonstrates that the onset of RB after formaldehyde inhalation is immediate, with a marked decrease in minute volume in mice and rats minutes after exposure begins. Because reduced respiration lessens exposure to an irritating chemical, the toxicity is reduced and the animal's survival is enhanced. This is important for the survival of rodents living in burrows and confined spaces that may not be able to avoid exposure. Figure A-18 (left panel) demonstrates that the effects of RB are reversible, but it can take several minutes to several hours for all physiological parameters to return to preexposure conditions, depending on the extent of hypothermia (Pauluhn, 2018; OECD, 2009; Pauluhn, 2008; Barrow et al., 1983; Jaeger and Gearhart, 1982).

The physiological signs of RB in rodents can be striking, but they are not signs of toxicity and, as such, are not considered appropriate for defining an animal POD. Also, the signs of RB are not relevant to humans since humans cannot experience RB. RB can only occur in small animals such as mice and rats that can, because of their small size, rapidly lower their core body temperatures when their metabolic rate reflexively decreases. Even a mild decrease in body temperature can lessen the toxicity and metabolic activation of many chemicals, but it can also slow the excretion of toxicants. Overall, the protection from cellular toxicity afforded by RB-induced hypothermia outweighs the undesirable effect of a slower excretion rate (Gordon et al., 2008). Even though RB has been reported in the literature since the 1960s, it is largely unknown to most toxicologists. None of the rodent inhalation studies of formaldehyde, except for a few RB-specific studies, attempted to identify or measure RB, including measures of body temperature and respiration. As RB likely occurred in most, if not all, rodent inhalation toxicity studies involving high level exposures to formaldehyde, this uncertainty is acknowledged and discussed in the assessment, and for particular health outcomes it is specifically considered during study evaluation (e.g., see description below regarding behavioral effects, since RB can affect activity).

Irritation, reflex bradypnea, and the RD_{50}: A test for assessing sensory irritation was developed by Yves Alarie in the 1960s. In an Alarie test, rodent respiration is measured before, during, and after exposure to one or more concentrations of an irritant, and then respiratory depression (RD) is statistically quantified. RD is followed by a subscript that gives the percentage of respiratory depression (e.g., RD_0, RD_{20}, RD_{50}, RD_{70}, etc.) The most commonly reported value in Alarie tests is the RD_{50}—the concentration of an irritating chemical that causes a 50% depression in the respiratory rate (Kane et al., 1979).

"Irritation" refers to two distinct processes. The first process is sensory irritation of nerve endings. URT irritation of the trigeminal nerve, which humans perceive as a burning or stinging sensation, is what triggers RB in rodents. The second process relates to an inflammatory response elicited by an irritating chemical, which is manifested by histopathologic changes such as local redness, edema, pruritus, and cellular alterations. Sensory irritation may prevent histopathologic damage through avoidance or through RB in rodents. Bos et al. (2002) found no correlation between chemical concentrations that cause sensory irritation (as measured by the Alarie test) and

concentrations that induce histopathological changes. For a variety of irritants, the lowest concentration that induces nasal histopathologic lesions can range from 0.3 times RD_{50} to more than 3 times RD_{50}.

Alarie tests are useful for (1) identifying chemicals which are URT sensory irritants, (2) quantifying irritating concentrations, and (3) ranking chemicals for their irritancy potential. Alarie (1981) proposed using 0.03 times RD_{50} values to predict threshold limit values (TLVs: typically used to define workplace exposures that can be repeatedly encountered without adverse effects) for a variety of irritants. More recently, Nielsen et al. (2007) proposed the use of animal RD_{50} and RD_0 values along with human data in a weight-of-evidence approach to predict acute or short-term TLVs, the RD_0 being a threshold or NOEL for decreased respiratory rate.

Tables A-16 and A-17 present formaldehyde RD values from several Alarie studies for mice and rats, respectively.[10] No RD values exist for female mice or rats. Across the literature, there is fairly good agreement on RD_{50} values for various strains of mice:

Table A-16. Formaldehyde respiratory depression (RD) values for several mouse strains and exposure durations

Study	Mouse strain	Exposure (min)	RD_{50} (mg/m^3)	RD_{10} (mg/m^3)	RD_0 (mg/m^3)
Kane and Alarie (1977)	♂ Swiss-Webster	10	3.8	0.5[a]	0.31[a]
Nielsen et al. (1999)	♂ BALB/c	10	4.9	0.4	
Barrow et al. (1983)	♂ B6C3F1	10	5.4	0.9*	0.49*
Chang et al. (1981)	♂ B6C3F1	10	6.0	–	–
de Ceaurriz et al. (1981)	♂ Swiss OF_1	5	6.5	–	–

[a]Value derived from a graph.

Figure A-20 shows that rats are less responsive to URT irritants than mice, which is why rats have higher RD_{50} values than mice:

Table A-17. Formaldehyde respiratory depression (RD) values for several rat strains and exposure durations.

Study	Rat strain	Exposure (min)	RD_{50} (mg/m^3)	RD_{10} (mg/m^3)	RD_0 (mg/m^3)
Cassee et al. (1996a)	♂ Wistar	30	12.3	–	–

[10]Several studies cited in Tables A-16 and A-17 tested formalin, which means the animals were co-exposed to formaldehyde and methanol. Considering that methanol's mouse RD_{50} of 54,963 mg/m^3 (41,514 ppm) is 10,000 times greater than formaldehyde's mouse RD_{50}, methanol was likely to have a negligible impact on the formaldehyde RD values (Nielsen et al., 2007).

Study	Rat strain	Exposure (min)	RD_{50} (mg/m^3)	RD_{10} (mg/m^3)	RD_0 (mg/m^3)
Barrow et al. (1983)	♂ F-344	10	16.1	1.2[a]	–
Gardner et al. (1985)	♂ Crl-CD	15	17.0	–	–
Chang et al. (1981)	♂ F-344	10	39.0	–	–

[a]Value derived from a graph.

Tolerance: Nearly all rodent studies that assessed RB are acute Alarie tests lasting no more than a few minutes or hours. There are no long-term studies that investigated whether-or-when rodents develop a tolerance to formaldehyde or other irritants and eventually begin to breathe normally. Mouse studies are a particular concern because mice have a greater RB response than rats and are able to sustain bradypnea and hypothermia for a longer period than rats. The bulleted short-term (4 days to 4 weeks) studies below examined the potential for rodents to develop tolerance to formaldehyde and cyfluthrin. The formaldehyde studies show no sign of tolerance over 10 days of exposure at concentrations as high as 18 mg/m^3, but what happens after 10 days remains unknown.

- Kane and Alarie (1977) observed a progressive decrease in respiratory rate (i.e., a progressively greater RB response) over 4 days of formaldehyde exposure in Swiss-Webster mice exposed to an RD_{50} of 3.8 mg/m^3. A similar lack of tolerance was also seen in mice exposed to acrolein (an aldehyde) at an RD_{50} of 3.9 mg/m^3.

- Chang et al. (1983) exposed mice and rats to 6.9 or 17.6 mg/m^3 formaldehyde (two of the concentrations used in the Battelle carcinogenicity study) 6 hours/day for 4 days. On day 4, both mice and rats showed concentration-related decreases in respiratory rate and minute volume, but the decreases in mice were markedly greater (see Figure A-20).

- Chang and Barrow (1984) observed no tolerance in F-344 rats exposed to 18 mg/m^3 formaldehyde for 10 days. Tolerance was observed in rats exposed over 4 days to a very high formaldehyde concentration of 34 mg/m^3, likely due to destruction or downregulation of sensory trigeminal nerve endings or receptors, respectively.

- Pauluhn (1998) exposed Wistar rats 6 hours/day, 5 days/week for 4 weeks to cyfluthrin, a pyrethroid URT irritant, at the acute RD_{50} concentration of 47 mg/m^3. Mean decreases in respiratory rate were 45% at week 2 and 55% at week 4, that is, there was no sign of tolerance. Since formaldehyde and cyfluthrin are both URT irritants, it is likely that similar results might be seen with formaldehyde.

Reflex bradypnea and interpreting health effects data: Current testing guidelines do not require examination of RB-related endpoints and reduced inhaled rodent exposure may complicate interpretations regarding inferences of potential human risk. For example, Battelle's carcinogenicity study illustrates an apparent role of RB in long-term studies. The study authors observed a disparity in formaldehyde-induced squamous metaplasia and inflammation between B6C3F1 mice and F-344 rats. Both species were identically exposed in whole-body chambers at

analytical concentrations of 0, 2.5, 6.9, or 17.6 mg/m^3. At comparable concentrations, nasal lesions were much less severe in mice than in rats. In fact, incidences of squamous cell carcinoma were similar in rats exposed at 6.9 mg/m^3 and in mice exposed at 17.6 mg/m^3—a difference in concentration of more than 2-fold (Kerns et al., 1983). Kerns et al. reasoned this 2-fold difference between mice and rats may be due to "their physiological responses to formaldehyde inhalation," that is, due to RB. To support their hypothesis, they cited a 4-day Alarie test by Chang et al. (1983, described in the bullet above, described in the bullet above) in which the reduction in minute volume was 2-fold greater in mice than in rats when exposed at 17.6 mg/m^3 (see Figure A-20). In other words, the rats exposed at 6.9 mg/m^3 and the mice exposed at 17.6 mg/m^3 may have had similar lesion incidences because they were exposed to approximately the same inhaled "dose" of formaldehyde due to RB.

The hypothesis offered by Kerns et al. (1983) that mice in the Battelle study inhaled about half as much formaldehyde as rats at 17.6 mg/m^3 due to RB, is logical and compelling, but there are no long-term RB data to support it at this time. Thus, although it might be considered appropriate to adjust a rodent POD to account for potential decreases in respiration (thus inferring that use of the exposure levels and corresponding results of that study may not be health protective for humans), this approach was not applied in this assessment. Overall, the lack of a long-term study to determine whether-or when rodents eventually develop tolerance to formaldehyde or any other URT irritant represents a significant data gap.

The potential impact of reflex bradypnea on behavioral studies: The normal physiological effects of RB can complicate the interpretation of behavioral studies in rodents. Hypothermia causes reduced peripheral nerve conduction velocity due to an apparent reduced flux of potassium and chloride ions across axon membranes. Hypothermia also causes prolonged synaptic delay time at neuromuscular junctions. A progressive decrease in body temperature results in ataxia, loss of fine motor control and reflexes, a reduction in cerebral blood flow and brain function, and eventually a loss of consciousness (OECD, 2009; Mallet, 2002). Thus, what appear to be chemically induced behavioral effects may actually be partly attributable to RB-induced hypothermia. Thus, the irritant effects were considered during evaluations of behavioral studies (see Section A.5.7), including a preference for studies that allowed for a recovery time of at least 2 hours after exposure before testing, given the recovery parameters discussed above.

The impact of reflex bradypnea on developmental toxicity studies: Pregnant dams are protected by RB, but their fetuses are not. Fetuses can experience developmental delays or defects due to impaired placental transfer of O_2 (hypoxia) and CO_2 (hypercapnia), fetal hypothermia, and malnutrition. Fetuses are more sensitive to the effects of hypothermia as compared to adults (OECD, 2009).

When dams experience RB, their fetuses may experience hypoxia due to (1) reduced maternal respiration and (2) a left shift in maternal oxyhemoglobin affinity caused by an increase in blood pH (respiratory alkalosis). Normal oxygen exchange to the fetus requires a gradient between

maternal and fetal oxyhemoglobin affinities. When pregnant dams experience RB, their blood pH becomes more alkaline, resulting in a left shift in maternal oxyhemoglobin affinity. A maternal left shift results in the affinities of maternal and fetal oxyhemoglobin being indistinguishable, which impairs oxygen exchange to the fetus (hypoxia) and removal of CO_2 (hypercapnia). Rossant and Cross (Rossant and Cross, 2001) describe hypoxia as a normal regulator of placental development in both humans and mice.

When Holzum et al. (1994[11]) exposed pregnant rats to cyfluthrin, they observed concentration-related decreases in fetal weights (see Figure A-21); Holzum et al. also observed concentration-related decreases in placental weights. Clearly, further studies on the impact of formaldehyde and other URT irritants on the placenta and fetus are needed, but the results of Holzum et al. show how RB has the potential to delay fetal growth. It should be noted that reductions in maternal feeding and metabolism during periods of RB can result in reduced fetal glucose levels. It is also important to emphasize that RB-induced developmental effects caused by fetal hypoxia, hypercapnia, hypothermia, and malnutrition are not relevant to humans.

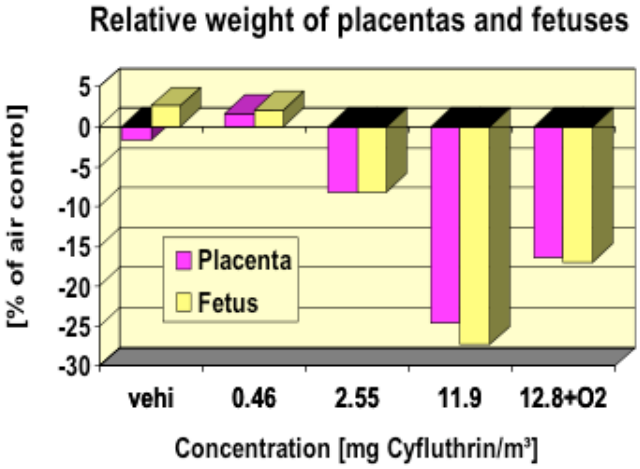


Figure A-21. The impact of Reflex Bradypnea on fetal development. This graph shows concentration-related decreases in placental and fetal weights in pregnant dams exposed to cyfluthrin, a pyrethroid insecticide. Note that the decrements in fetal and placental weights were lessened in the 12.8 mg/m^3 group when the dams were provided with oxygen-rich air (39% O_2).

Source: Holzum et al. (1994). Graph generated by Jűrgen Pauluhn (Bayer Healthcare AG, Germany).

Summary: Reflex bradypnea (RB) is a protective response observed in rodents exposed to formaldehyde and other upper respiratory tract irritants. The most notable signs of RB are concentration-related decreases in body temperature, respiratory rate (breaths/minute), and

[11] https://www3.epa.gov/pesticides/chem_search/cleared_reviews/csr_PC-128831_13-Feb-01_b.pdf

minute volume. Even though the effects of RB can be striking, they are not relevant to humans. It is likely that RB occurred in most, if not all, rodent inhalation toxicity studies testing high levels of formaldehyde exposure, but the extent of RB in these studies cannot be ascertained since it was not measured. In comparative studies, mice exhibit RB at a lower formaldehyde concentration than rats and had a more pronounced and more sustained RB response than rats.

Because rodents experiencing RB have reduced minute volumes, they inhale less formaldehyde and thus are expected to experience less toxicity than if they were breathing normally. Several studies demonstrate that mice and rats do not develop tolerance to formaldehyde over as much as 10 days of exposure; however, there are no long-term studies that show whether-or-when rodents eventually develop a tolerance to formaldehyde. This is a significant data gap. Thus, while RB is considered during study evaluation and during evidence synthesis and integration, adjustments are not applied to account for the potential impact of RB on long-term rodent health endpoints considered for use in dose-response analysis.

A.4. GENOTOXICITY

The evaluations of genotoxic effects of formaldehyde exposure included primary sources from peer-reviewed literature and secondary sources of peer-reviewed reports by other federal agencies and non-federal institutions (see Section A.4.7), although a systematic literature search was not conducted. In general, the following criteria were considered for making judgments about evidence for the genotoxic and/or mutagenic potential of formaldehyde. These include but are not limited to: (a) nature and type of tests, (b) degree of response, (c) number and performance of test strains, (d) dose/concentration levels, (e) biological significance, (f) strength of evidence (conflicting evidence in the same assay system for the same end point), and (g) evaluation of the study results across the same end points. Studies of genotoxicity in exposed humans were consistently evaluated using a structured set of criteria (see Section A.4.7).

The terms genotoxicity and mutagenicity differ depending on the effect seen on DNA. Genotoxicity refers to potentially harmful effects caused either directly or indirectly to the genetic material by chemical or physical agents, and these effects are not necessarily persistent and transmissible and may or may not be associated with mutagenicity. Mutagenicity refers to the induction of permanent, transmissible changes in the amount, chemical properties, or structure of the genetic material. Mutations may involve a single gene or gene segment, a block of genes, parts of chromosomes, or whole chromosomes and result in either structural and/or numeric changes. Since mutagenicity is considered a subset of gentoxic effects, the term "genotoxic effects" will be generally used through out the rest of the document unless the assay determines specific mutations.

A variety of genotoxic effects have been demonstrated in both in vitro and in vivo test systems as a result of exposure to formaldehyde (a Summary Table by Genotoxic Endpoint is presented in Section A.4.7). Note that no single genotoxicity or mutagenicity test/system or study

is able to detect the entire spectrum of formaldehyde-induced genotoxic events. Therefore, genotoxic endpoints are briefly discussed for cell free systems, prokaryotic organisms, nonmammalian organisms, in vitro mammalian systems, in vivo experimental animals, and humans [reviewed in (NTP, 2010; ATSDR, 2008; IARC, 2006; Liteplo and Meek, 2003; Conaway et al., 1996; IARC, 1995; Ma and Harris, 1988; Auerbach et al., 1977). In addition, the overall weight of evidence for formaldehyde-induced mutations is considered in the context of the current EPA cancer guidelines (U.S. EPA, 2005). Note that all studies from the available database have been depicted in several of the following tables, but only the studies most relevant to this discussion are briefly described in the text.

A.4.1. Genotoxicity of Formaldehyde in Cell-Free Systems

Formaldehyde or formalin[12] has been shown to form both hydroxymethyl DNA (hmDNA) adducts and DNA-protein crosslinks (DPX) following treatment of various cell-free systems with formaldehyde or formalin (see Table A-18). The formation of DNA-DNA crosslinks were observed in calf thymus DNA (Chaw et al., 1980) and duplex DNA (Huang and Hopkins, 1993; Huang et al., 1992). Furthermore, DNA-protein crosslinks were seen in plasmid DNA, calf thymus histones, and other acelluar systems (Lu et al., 2010b; Lu, 2009; Lu et al., 2008; Kuykendall and Bogdanffy, 1992). The formation of hmDNA adducts was observed following in vitro reaction of formalin in solution with free DNA ribonucleoside (Kennedy et al., 1996), deoxyribonucleosides and nucleotides (Cheng et al., 2008; Cheng et al., 2003; Mcghee and von Hippel, 1975a, b), calf thymus DNA (Fennell, 1994; Beland et al., 1984; Von Hippel and Wong, 1971), human placental DNA (Zhong and Hee, 2004), and isolated rat liver nuclei (Fennell, 1994; Heck and Casanova, 1987). Cheng et al. (2008) also reported that nitrosamines which form formaldehyde during their metabolism via formation of α-esters can react in vitro with deoxyribonucleosides or calf thymus DNA and form the hmDNA adducts. Studies have shown that N^6-hydroxymethyl-deoxyadenosine (N^6-hmdAdo) was the predominant adduct formed followed by N^2-hydroxymethyl-deoxyguanosine (N^2-hmdGuo) and N^4-hydroxymethyl-deoxycytidine (N^4-hmdCyd) when formaldehyde was reacted with calf thymus DNA (Cheng et al., 2008; Beland et al., 1984) or human placental DNA (Zhong and Hee, 2004).

Table A-18. Summary of genotoxicity of formaldehyde in cell-free systems

Test system	Dose and Agent[a]	Results[b]	Duration; Method	Reference
DNA-DNA crosslinks				
Calf thymus DNA	0.17 mM 37% HCHO	+	40 d; RP-HPLC	Chaw et al. (1980)

[12]Studies that used formalin often contained 10-15% methanol as a stabilizing agent. Although formaldehyde is a metabolic product of methanol, it is not genotoxic in in vitro reactions.

Test system	Dose and Agent[a]	Results[b]	Duration; Method	Reference
Duplex DNA	25 mM HCHO	+	9 d; DPAGE	Huang et al. (1992)
Duplex DNA	25 mM HCHO	+	9 d; DPAGE	Huang and Hopkins (1993)
DNA-protein crosslinks				
Lysine or Cysteine and dG	50 mM 20% HCHO in H_2O	+	48 hrs; RP-HPLC/LC_MS	Lu et al. (2010a)
Histone 4	50 mM 20% HCHO in H_2O	+	10 min; LC-MS	Lu et al. (2008)
Plasmid DNA, calf thymus histones	0.0015 mM HCHO	+	1 hr; filter binding assay	Kuykendall and Bogdanffy (1992)
Calf thymus DNA	0.5 mM HCHO	+	4 hrs; ESI-MS/MS	Lu (2009)
DNA adducts				
Guanosine	2,400 mM 37% HCHO	+	48 hrs	Kennedy et al. (1996)
Deoxyguanosine	2,300 mM formalin[c]	+	20 hrs	Cheng et al. (2003)
Guanosine	0.001 mM HCHO	+	90 hrs	Cheng et al. (2003)
DNA nucleosides/ nucleotides	50 mM formalin	+	72–120 hrs	Mcghee and von Hippel (1975a)
DNA nucleosides/ nucleotides	300 mM formalin	+	72–120 hrs	Mcghee and von Hippel (1975a)
Calf thymus DNA	0.001 mM formalin	+	90 hrs	Cheng et al. (2003)
Calf thymus DNA	0.167 mM formalin	+	48 hrs	Beland et al. (1984)
Calf thymus DNA	0.4 mM formalin	+	4 hrs	Fennell (1994)
Calf thymus DNA	200 mM formalin	+	20 hrs	Von Hippel and Wong (1971)
Calf thymus DNA or deoxyribonucleosides	50 mM α-acetates of NDMA; NNK and NNAL[d]	+	1 or 90 hrs	Cheng et al. (2008)
Human placental DNA	3.34 mM formalin	+	20 hrs	Zhong and Hee (2004)
Rat - Hepatic nuclei	0.1 mM HCHO (^{14}C and ^{3}H) aqueous solution	+	0.5 hr	Heck and Casanova (1987)

Test system	Dose and Agent[a]	Results[b]	Duration; Method	Reference
Rat - Hepatic nuclei	0.4 mM ^{14}C-HCHO	+	4 hrs	Fennell (1994)

[a]lowest effective concentration for positive results; highest concentration tested for negative or equivocal results.
[b]+ = positive, all experiments performed without exogenous activation.
[c]Formalin − all experiments with formalin contained 37% formaldehyde plus 10–15% methanol.
[d]these nitrosamines are precursors to formaldehyde.
Abbreviations: HCHO, formaldehyde; NDMA, N-nitrosodimethylamine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; DPAGE, denaturing polyacrylamide gel electrophoresis; HPLC, high performance liquid chromatography; LC-ESI-MS, liquid chromatography electrospray ionization mass spectrometry; LSC, liquid scintillation counting; MS, mass spectrometry; NMR, nuclear magnetic resonance; RP-HPLC, reverse phase high performance liquid chromatography; UV, ultraviolet.

A.4.2. Genotoxicity of Formaldehyde in Prokaryotic Organisms

A number of reports describe the mutagenicity of formaldehyde in bacterial test systems (*Salmonella typhimurium* and *Eschericia coli*) using reverse and forward mutation assays as well as assays with specific *E. coli* strains for detecting deletions, insertions and point mutations (see Table A-19).

Formaldehyde was mutagenic in the reverse mutation assay in all of the studies with the Salmonella strains TA102 and TA104, and most of the studies with TA100 strains with and without metabolic activation and in strains TA2638 and TA2638a without metabolic activation. Mixed results were reported with TA97, TA98, and TA1537 strains, while most of the studies with the TA1535 and TA1538 strains were negative with or without metabolic activation (Rydén et al., 2000; Dillon et al., 1998; Sarrif et al., 1997; Le Curieux et al., 1993; Müller et al., 1993; O'Donovan and Mee, 1993; Jung et al., 1992; Wilcox et al., 1990; Marnett et al., 1985).

With respect to forward mutations, formaldehyde has been shown to induce these types of mutations both in *S. typhimurium* (Temcharoen and Thilly, 1983) as well as in *E. coli* strains (Bosworth et al., 1987; Temcharoen and Thilly, 1983). Temcharoen and Thilly (1983) showed that formaldehyde induced both toxicity and mutagenicity in the Salmonella strain TM677 (8-azaguanine sensitive), both with or without metabolic activation. On the other hand, Bosworth et al. (1987) reported formaldehyde to be mutagenic in *E. coli* strain D494 uvrB, a more sensitive strain to base-pair substitutions. Furthermore, formaldehyde has been shown to induce diverse mutations in a forward mutation assay in *E. coli* strains GP120, GP120A, 7-2, and 33694, which contained a xanthine guanine phosphoribosyl transferase (*gpt*) reporter gene (Crosby et al., 1988). In this study, formaldehyde tested at two different concentrations (4 and 40 mM) produced point mutations (41%), deletions (18%), and insertions (41%) at low concentrations of exposure, while the high-dose exposure resulted predominantly in point mutations (92%). The point mutations at low-dose exposure were transversions at GC base pairs, while at high-dose exposure they were transition mutations at a single AT base pair in the *gpt* gene (Crosby et al., 1988).

Wang et al. (2007b) have also shown that formaldehyde causes dose-dependent increase in microsatellite instability in *E. coli*. Exposure to 2.5 mM formaldehyde caused a 2- to 24-fold

induction in mutation frequencies of the complementary dinucleotide repeat microsatellites (GpT) and (ApC) compared to in untreated controls. It is possible that microsatellite instability could change the conformation of DNA to Z-DNA structure, making the DNA not amenable for DNA repair.

Table A-19. Summary of genotoxicity of formaldehyde in prokaryotic systems

Test system	Dose[a] (µg/plate)	Agent[b]	Results[c,d]		Comments	Reference
			−S9	+S9		
Reverse mutation						
S. typhimurium TA100	10, 25	35% HCHO sol.	+	+	PP method; values visually determined from graph; (T) at 37.5 (−S9) and 50 (+S9) µg/plate	Orstavik and Hongslo (1985)
	12	37% HCHO with 10% methanol	(+)	(+)	PI method	Schmid et al. (1986)
	15, 7.5	HCHO/mL	+	+	Suspension method	Sarrif et al. (1997)
	30	37% HCHO with 10−15% methanol	+	+	PI method; values visually determined from graph. Methanol tested '−ve' up to 500 µg/plate (−S9 or +S9) in the same study.	Connor et al. (1983)
	30	HCHO (form not specified)	(+)	ND	PP method	Takahashi et al. (1985)
	39	37% HCHO with 10−15% methanol	− (T)	− (T)	PI method	De Flora (1981)
	50	35% HCHO	+	+	PP method; dose range 6.25-50 µg/plate only provided	Dillon et al. (1998)
	75	HCHO (form not specified)	−	+	PI method; −S9 data <2-fold compared to control	Sarrif et al. (1997)
	80	37% HCHO with 10% methanol	(+)	+	PP method	Schmid et al. (1986)
	90	HCHO (form not specified)	−	ND	PP method; (T): >90 µg/plate	Marnett et al. (1985)
	100, 50	37% aq.sol. HCHO	+, +	ND	Results by PI & PP methods, respectively	O'Donovan and Mee (1993)
	100	HCHO (form not specified)	+	−	PP method; (T) ≥200 µg/plate	Sarrif et al. (1997)
	150	37% HCHO	+	ND	PP method; Discrepancy in	Fiddler et al. (1984)

Test system	Dose[a] (µg/ plate)	Agent[b]	Results[c,d]		Comments	Reference
			−S9	+S9		
					mutagenic data observed between author's report and the graph from the citation (150 vs. ≈30 µg/plate)	
	333.3, 10	37% HCHO	−	+	PP method; (T): NR	Haworth et al. (1983)
	500, 20	37% HCHO in distilled water	(+)	+	PP method	Connor et al. (1985a)
S. typhimurium TA102	10	HCHO/mL	+	ND	Fluctuation test; (T) at 30 µg/mL	Le Curieux et al. (1993)
	17.2	HCHO (in water)	+	ND	PP method	Rydén et al. (2000)
	25	HCHO (form not specified)	+	ND	PI method; (T) >100 µg/plate	Wilcox et al. (1990)
	50	HCHO (form not specified)	(+)	(+)	PP method; values visually determined from graph	De Flora et al. (1984)
	50	35% HCHO	+	+	PP method; '+' with rat S9 and '±' with mouse S9; Authors show a dose range 6.25–50 µg/plate.	Dillon et al. (1998)
	90	HCHO (form not specified)	+	ND	PP method; (T): >90 µg/plate	Marnett et al. (1985)
	200, 100	37% aq.sol. HCHO	+, +	ND	Results by PI & PP methods, respectively	O'Donovan and Mee (1993)
	200	HCHO (in water)	+	ND	PI method; (T) at 600 mg/plate	Watanabe et al. (1996)
	5000	HCHO (form not specified)	(+)	(+)	PI method; (+) by 1 lab and '−ve' by 2 labs	Jung et al. (1992)
	5,000	HCHO (form not specified)	(+)	(+)	PI method; reported '(+) by one lab and '−ve' by 2 labs	Müller et al. (1993)
S. typhimurium TA104	50	35% HCHO	+	+	PP method; Authors show a dose range 6.25–50 µg/plate.	Dillon et al. (1998)
	90	HCHO (form not specified)	+	ND	PP method; (T): >90 µg/plate	Marnett et al. (1985)
S. typhimurium TA1535	39	formalin	− (T)	− (T)	PI method	De Flora (1981)
	100	37% aq.sol. HCHO	−, −	ND	Results by PI & PP	O'Donovan and

Test system	Dose[a] (µg/ plate)	Agent[b]	Results[c,d]		Comments	Reference
			−S9	+S9		
					methods, respectively	Mee (1993)
	100	HCHO (form not specified)	–	–	PI method; (T) at 150 µg/plate	Sarrif et al. (1997)
	100	HCHO (form not specified)	–	–	PP method; (T) ≥200 µg/plate	Sarrif et al. (1997)
	333.3	37%HCHO	–	–	PP method; (T): NR	Haworth et al. (1983)
S. typhimurium TA97	50	HCHO (form not specified)	+	ND	PI method; (T) at 100 µg/plate	Sarrif et al. (1997)
	90	HCHO (form not specified)	–	ND	PP method; (T): >90 µg/plate	Marnett et al. (1985)
S. typhimurium TA98	10, 25	35% HCHO sol.	+	+	PP method; values visually determined from graph; (T) at 37.5 (−S9) and 50 (+S9) µg/plate	Oerstavik and Hongslo (1985)
	30	37% HCHO with 10-15% methanol	+	+	PI method; Methanol tested up to 500 mg/plate (−S9 or +S9) was '−ve'. Values visually determined from graph.	Connor et al. (1983)
	30	HCHO (form not specified)	(+)	ND	PP method	Takahashi et al. (1985)
	39	37% HCHO with 10-15% methanol	– (T)	– (T)	PI method	De Flora (1981)
	50, 100	37% aq.sol. HCHO	+, +	ND	Results by PI & PP methods, respectively	O'Donovan and Mee (1993)
	50, 100	HCHO (form not specified)	+	+	PP method; (T) ≥00 µg/plate	Sarrif et al. (1997)
	75	HCHO (form not specified)	–	+	PI method; −S9 data <2-fold compared to control	Sarrif et al. (1997)
	90	HCHO (form not specified)	–	ND	PP method; (T): >90 µg/plate	Marnett et al. (1985)
	333.3, 10	37% HCHO	–	(+)	PP method; (T): NR	Haworth et al. (1983)
	500	37% HCHO in distilled water	– (T)	(+) (T)	PP method	Connor et al. (1985b)
S. typhimurium TA1537	39	37% HCHO with 10-15% methanol	– (T)	– (T)	PI method	De Flora (1981)

Test system	Dose[a] (µg/plate)	Agent[b]	Results[c,d]		Comments	Reference
			−S9	+S9		
	50, 75	HCHO (form not specified)	+	+	PI method	Sarrif et al. (1997)
	100	37% aq.sol. HCHO	−, −	ND	Results by PI & PP methods, respectively	O'Donovan and Mee (1993)
	100	HCHO	−	−	PP method	Sarrif et al. (1997)
	333.3	37% HCHO	−	−	PP method; (T): NR	Haworth et al. (1983)
S. typhimurium TA1538	39	formalin	− (T)	− (T)	PI method	De Flora (1981)
	100	37% aq.sol. HCHO	−, −	ND	Results by PI & PP methods, respectively	O'Donovan and Mee (1993)
S. typhimurium TA2638	500	HCHO (in water)	+	ND	PI method; (T) at 1000 mg/plate	Watanabe, 1996, 626156@@author-year}
S. typhimurium TA2638a	17.2	HCHO (in water)	+	ND	PP method	Rydén et al. (2000)
S. typhimurium UTH8413, UTH8414	500	37% HCHO with 10−15% methanol	− (T)	− (T)	PI method; Methanol tested '−ve' up to 500 µg/plate with/without S9.	Connor et al. (1983)
	500	37% HCHO in distilled water	− (T)	− (T)	PP method	Connor et al. (1985b)
E. coli WP2, WP2uvrA, H/R30R, Hs30R (uvrA)	420	HCHO (form not specified)	+	ND	RM assay	Takahashi et al. (1985)
E. coli NG30 (recA)	63	HCHO (form not specified)	−	ND	RM assay; values visually determined from graph	Takahashi et al. (1985)
E. coli O16 (polA)	52.5	HCHO (form not specified)	−	ND	RM assay; values visually determined from graph	Takahashi et al. (1985)
E. coli K12 (AB1886)/(uvrA); K12 (AB2480)/(recA/uvrA)	150	HCHO (form not specified)	−	ND	RM assay	Graves et al. (1994)
E. coli K12 (AB1157)(WT)	1,875	HCHO (form not specified)	+	ND	RM assay	Graves et al. (1994)
E. coli WP2 (pkM101)	200	HCHO (form not specified)	− (T)	ND	PI method	Wilcox et al. (1990)
	200, 100	37% aq.sol. HCHO	−, +	ND	Results by PI & PP methods, respectively	O'Donovan and Mee (1993)
	700	HCHO (in water)	+	ND	PI method	Watanabe et al. (1996)

Test system	Dose[a] (µg/plate)	Agent[b]	Results[c,d]		Comments	Reference
			−S9	+S9		
E. coli WP2 uvrA (pkM101)	150	HCHO (form not specified)	+	ND	PI method; dose-response from 10−300 µg/plate	Wilcox et al. (1990)
	200, 50	37% aq.sol. HCHO (form not specified)	+, +	ND	Results by Results by PI & PP methods, respectively	O'Donovan and Mee (1993)
	400	HCHO (in water)	+	ND	PI method	Watanabe et al. (1996)
E. coli (Lac+ reversion) WP3104P	10	HCHO (form not specified)	(+)	ND	RM assay	Ohta et al. (1999)
E. coli (Lac+ reversion) WP3101P, WP3102P, WP3103P, WP3105P, WP3106P	30	HCHO (form not specified)	−	ND	RM assay	Ohta et al. (1999)
Forward mutation						
S. typhimurium TM677	0.167, 0.33 mM	37% HCHO with 10−15% methanol	+	+	PP method	Temcharoen and Thilly (1983)
E. coli D494*uvr*B (pGW1700)	6.0 µg/mL	HCHO (form not specified)	+	ND	Ampicillin FM assay	Bosworth et al. (1987)
Deletions, Insertions and Point mutations						
E. coli GP120, GP120A, 7-2, 33694	4 mM	HCHO (form not specified)	+	ND	*gpt* FM assay	Crosby et al. (1988)
Microsatellite Instability						
E. coli JM109	2.5 mM	HCHO (form not specified)	+	ND	Mutation frequency analysis and sequencing.	Wang et al. (2007b)

[a]lowest effective dose for positive results; highest ineffective dose tested for negative or equivocal results.
[b]single value indicates identical dose/concentration effective for both without (−S9) or with (+S9) metabolic activation; for −S9 assay data showing two signs (+ or -) separated by a comma indicate respectively, use of PI and PP methods.
[c]+ = positive; − = negative; (+) = weak positive; ND = test was not done; (T), toxic.
Abreviations: HCHO, formaldehyde; PI, plate incorporation (or standard plate); PP, pre-incubation plate; FM, forward mutation; RM, reverse mutation; *gpt*, xanthine guanine phosphoribosyl transferase.

A.4.3. Genotoxicity of Formaldehyde in Nonmammalian Systems

Formaldehyde (commercial grade) or formalin (mostly containing 37% formaldehyde and 10−15% methanol) has been tested in several nonmammalian systems including yeast, molds, plants, insects, and nematodes. As summarized in Table A-20, formaldehyde has been shown to cause gene conversion, strand breaks, crosslinks, homozygosis and related damage in yeasts (*Saccharomyces cerevisiae*); forward and reverse mutations in molds (*Neurospora crassa*); micronuclei formation in spiderworts (*Tradescantia pallida*); DNA damage and mutations in several

plants; genetic cross-over or recombination, sex-linked recessive lethal mutations, dominant lethal mutations, heritable translocations, and gene mutations in insects (*Drosophila melanogaster*); and recessive lethal mutations in nematodes (*Caenorhabditis elegans*). Formaldehyde failed to show micronuclei formation in newt larvae (*Pleurodeles waltl*) (reviewed in reviewed in IARC, 2012; NTP, 2010; IARC, 2006). DNA protein crosslinks were observed in *Saccaromyces cerevisiae* and *E. coli* (Magaña-Schwencke and Moustacchi, 1980; Magaña-Schwencke and Ekert, 1978; Wilkins and Macleod, 1976).

Some of the nonmammalian studies compared the effects of formaldehyde in wild type and DNA repair-deficient organisms. For example, Magaña-Schwencke et al. (1978) showed that excision repair-deficient *Saccharomyces cerevisiae* strains are more susceptible to formaldehyde-induced lethal effects and have reduced capacity to form single strand breaks (SSBs) compared with repair-proficient strains, suggesting that the repair process possibly involves SSB formation. Also, formaldehyde is more mutagenic in repair-deficient *Neurospora crassa* compared to the corresponding repair-proficient strains (de Serres and Brockman, 1999).

Table A-20. Summary of genotoxicity studies for formaldehyde in nonmammalian organisms

Test system	Concentration[a,b]	Results[c]	Comments[d]	Reference
DNA damage				
Various plant and fungal species[e]	1233 mM 3.7% HCHO (at pH 3.0 and 7.0)	+	1.5 hrs, PCR/GE,	Douglas and Rogers (1998)
DNA protein crosslinks				
Saccharomyces cerevisiae	17 mM HCHO (form not specified)	+	0.25 hrs, DNA extractability; (T) 90 & 60% survival at 33 & 66 mM HCHO with 42 & 95% DNA damage, respectively	Magaña-Schwencke and Ekert (1978)
S. cerevisiae	33 mM HCHO (form not specified)	+		Magaña-Schwencke and Moustacchi (1980)
E. coli	130 mM HCHO (form not specified)	+	10 min; alkaline sucrose gradient centrifugation	Wilkins and Macleod (1976)
DNA repair inhibition				
S. cerevisiae	66 mM HCHO (form not specified)	+	0.25 hrs, ASG; (T) 90 & 60% survival at 33 & 66 mM HCHO with 42 & 95% DNA damage, respectively	Magaña-Schwencke and Ekert (1978)
Dominant lethal mutation				
Drosophila melanogaster	60 mM 36% HCHO in water	+	larval feeding method, frequency of hatchability	Auerbach and Moser (1953a, 1953b)
D. melanogaster	43 mM HCHO (form not specified)	+	Exposure duration NR, frequency of dominant lethal mutations	Srám (1970)
Forward mutation				

Test system	Concentration[a,b]	Results[c]	Comments[d]	Reference
Neurospora crassa heterokaryon H-59 strain	3 mM formalin	+	3 hrs, frequency of ad-3 mutations	de Serres and Brockman (1999); de Serres et al. (1988)
N. crassa heterokaryon H-12 strain	8 mM formalin	(+)	3 hrs, frequency of ad-3 mutations	de Serres and Brockman (1999); de Serres et al. (1988)
Gene conversion				
S. cerevisiae strain D4	18 mM 30% HCHO solution	+	0.5 hr, frequency of recombinants	Chanet et al. (1975)
Genetic crossing over or recombination				
D. melanogaster	14 mM HCHO (form not specified)	+	larval feeding method	Alderson (1967)
	42 mM HCHO (form not specified)	+	duration of exposure NR, frequency of recombinant	Sobels and van Steenis (1957)
	83 mM HCHO (form not specified)	+	duration of exposure NR, frequency of cross overs	Ratnayake (1970)
Heritable translocation				
D. melanogaster	14 mM HCHO (form not specified)	+	2 hrs, frequency of recombinants	Khan (1967)
	83 mM HCHO (form not specified)	+	duration of exposure NR, frequency of translocations	Ratnayake (1970)
Homozygosis by mitotic recombination or gene conversion				
Saccharomyces cerevisiae	0.62 mM formalin	+	16 hrs, frequency of resistant colonies	Zimmermann and Mohr (1992)
Micronucleus				
Pleurodeles waltl	0.17 mM HCHO (form not specified)	−	168 hrs, Masson's haemalum staining	Siboulet et al. (1984)
Pleurodeles waltl larva	0.33 mM HCHO (form not specified)	−	12 hrs, Masson's haemalum staining	Le Curieux et al. (1993)
Tradescantia pallida	8 mM HCHO (form not specified)	+	6 hrs, acetocarmine staining	Batalha et al. (1999)
Mutation				
Plants (others)	NR	+	NR	Auerbach et al. (1977)
Reverse lethal mutation				
Caenorhabditis elegans	23 mM HCHO from PFA	+	4 hrs, frequency of mutations	Johnsen and Baillie (1988)
Reverse mutation				

Test system	Concentration[a,b]	Results[c]	Comments[d]	Reference
Neurospora crassa	10 mM HCHO (form not specified)	+	4 hrs, frequency of mutations	Jensen et al. (1951)
	10 mM formalin	–	3 hrs, frequency of mutations	Kölmark and Westergaard (1953)
	24 mM HCHO (form not specified)	–	0.5 hrs, frequency of mutations	Dickey et al. (1949)
Sex-linked lethal mutation				
D. melanogaster	8 mM formalin	+	larval feeding method, frequency of sex linked lethals	Stumm-Tegethoff (1969)
	14 mM HCHO (form not specified)	+	larval feeding method	Alderson (1967)
	14 mM HCHO (form not specified)	+	2 hrs, frequency of progeny	Khan (1967)
	33 mM formalin	+	duration of exposure NR, frequency of eclosions	Kaplan (1948)
	42 mM HCHO (form not specified)	+	Exposure duration NR, frequency of sex-linked lethals	Sobels and van Steenis (1957)
	60 mM 36% HCHO in water	+	larval feeding method, frequency of sex linked lethals	Auerbach and Moser (1953b)
	67 mM HCHO (form not specified)	(+)	larval feeding method, frequency of sex linked lethals	Ratnayake (1968)
	73 mM HCHO (form not specified)	+	duration of exposure NR, frequency of sex-linked lethals	Ratnayake (1970)
Single strand breaks				
S. cerevisiae	33 mM HCHO (form not specified)	+	0.25 hrs, ASG; (T) 90 & 60% survival at 33 & 66 mM HCHO with 42 & 95% DNA damage, respectively	Magaña-Schwencke et al. (1978)

[a]indicates lowest effective concentration for positive results; highest concentration tested for negative or equivocal results.

[b]indicates that the multiple dose/concentration values reported correspond to order of the indicated test result(s) (e.g., without activation; with activation). Identical doses/concentrations for multiple test results are indicated by a single value.

[c]indicates + = positive; – = negative; (+) = weak positive.

[d]indicates the duration of exposure and the assay used to assess the endpoint, dose-response and toxicity (T) if any.

[e]indicates that authors tested the following species: *Agaricus bisporus*, *Glycine max*, *Lycopersicon esculentum*, *Pinus resinosa*, *Pisum sativum*, *Populus* x *euramericana*, *Vicia faba*, and *Zea mays.*

Abbreviations: ad-3, adenine-3 locus; ASG, alkaline sucrose gradient; HCHO, formaldehyde; NR, not reported; PCR/GE, polymerase chain reaction/gel electrophoresis; PFA, paraformaldehyde.

A.4.4. Genotoxicity of Formaldehyde in in Vitro Mammalian Cells

Formaldehyde has been tested for its genotoxic potential in several mammalian cell culture systems originating from rodents (mice, rats, hamsters) and humans, mostly without metabolic activation. In a majority of these systems, formaldehyde tested positive for: DNA reactivity including DNA adducts, DPXs, and SSBs; cytogenetic changes such as sister chromatid exchanges (SCEs), chromosomal aberrations (CAs), and micronuclei (MN); cell transformation and mutation induction; and other genotoxic endpoints such as unscheduled DNA synthesis (UDS) and DNA repair inhibition (summarized in Table A-21).

DNA Reactivity and Damage

DNA adducts

Formaldehyde has been shown to form hmDNA adducts in CHO cells (Beland et al., 1984) and rat and human nasal epithelial cells (Zhong and Que Hee, 2004) as shown in Table A-21. Beland et al. (1984) first reported hmDNA adducts in CHO cells incubated with 1 mM of radiolabeled formaldehyde. After a 2-hour incubation, small amounts of N^6-hmdA were detected with concomitant metabolic incorporation of formaldehyde (i.e., into DNA bases). Zhong and Que Hee (2004) reported three types hmDNA adducts in human nasal epithelial cells exposed to varying concentrations of formalin (10–500 µg/mL). In this study, the hmDNA adduct levels were in the order of N^6-hmdA > N^2-hmdG > N^4-hmdC. In HeLa cells exposed to [$^{13}CD_2$]-formaldehyde, Lu et al. (2012a) detected both exogenous (^{13}C-labeled) and endogenous (unlabeled) N^2-hmdG adducts; however, this study detected endogenous but not exogenous N^6-hmdA adducts.

DNA-protein crosslinks

As summarized in Table A-21, DNA protein crosslinks have been reported in several mammalian cell lines (primary and transformed) from rodents (mice, rats, hamsters) and humans. (reviewed in reviewed in IARC, 2006; Conaway et al., 1996; IARC, 1995).

The lowest effective concentration of formaldehyde or formalin causing DPX formation varied between different cell lines (see Table A-21). Among the animal cell lines, DPX formation was observed at the in vitro concentrations of 0.125–0.25 mM in CHO cells and 0.01–0.2 mM in V79 cells. Several human cell lines (either primary cells or developed cells lines), including epithelial, fibroblasts, buccallymphoblastoid, lymphoma, and peripheral blood lymphocytes, among others, that were exposed to formaldehyde also formed DPXs (Emri et al., 2004; Li et al., 2004; Costa et al., 1997; Craft et al., 1987). Selected studies have been briefly described below, although all available and relevant studies are included in Table A-21).

Craft et al. (1987) analyzed DPXs in TK6 human lymphoblastoid cells immediately after a 2-hour exposure (zero time) to 0–600 µM formaldehyde with a significant nonlinear increase in DPXs above 50 µM, which correlated with the onset of cytotoxicity. DPXs were completely repaired within 24 hours after exposure.

DPXs were also detected in Epstein-Barr Virus (EBV)-human Burkitt's lymphoma cells exposed to paraformaldehyde (which depolymerizes to release formaldehyde) at doses that were cytotoxic (>0.003%) (Costa et al., 1997). Grafström et al. (1986) reported that the number of DPXs induced by 100 μM formaldehyde in vitro in human bronchial epithelial cells and fibroblasts was similar; although, DPX levels were several-fold higher than SSBs in the epithelial cells. In a different study, the same authors (Grafstrom et al., 1984) noted that formaldehyde exposure resulted in the formation of DPXs at similar levels in bronchial epithelial cells and in DNA excision repair-deficient xeroderma pigmentosum (XP) skin fibroblasts, and their removal rate was similar with a half-life of 2–3 hours, suggesting that the DPX are repaired independently of the excision repair. Further, formaldehyde was only moderately cytotoxic to normal bronchial epithelial cells and fibroblasts at concentrations that induced substantial DNA damage. Repair of the formaldehyde-induced DNA SSBs and DPXs appeared to be inhibited by the continued presence of formaldehyde in the culture medium (Grafstrom et al., 1984).

A linear increase in DPX levels was observed in primary human skin fibroblasts and keratinocytes from 25–100 μM formaldehyde, as indicated by the ability of formaldehyde to reduce DNA migration in the comet assay after methylmethane sulfonate (MMS) pretreatment (Emri et al., 2004). Similar findings were also reported for primary human peripheral blood lymphocytes (PBLs) and HeLa cells (LICM, 2006). Peak response for SSBs was seen at 10 μM in both cells, with higher concentrations resulting in crosslink formation (LICM, 2006). DPX formation was also observed in whole blood culture after exposure to 25 μM, as indicated by the affect of formaldehyde on DNA migration in the comet assay after γ–radiation (Schmid and Speit, 2007). The repair of DPX was complete 8 hours after an exposure to 100 μM formaldehyde, while DPX formed at >200 mM were repaired within 24 hours.

Formaldehyde-induced DPXs are removed either through spontaneous hydrolysis or active repair processes (Quievryn and Zhitkovich, 2000). Inhibition of specific proteosomes (protein complexes involved in degrading unwanted or damaged proteins) in xeroderma pigmentosum (XP)-A cells inhibited DPX repair, thereby supporting the role of enzymatic degradation (Quievryn and Zhitkovich, 2000). The average half-life of formaldehyde-induced DPXs in human epithelial cell lines was 12.5 hours (range 11.6 to 13 hours) (Quievryn and Zhitkovich, 2000), 18 hours in HeLa cells (LICM, 2006), and 24 hours in human lymphoblasts (Craft et al., 1987). This difference was primarily due to slower active repair of DPXs, with a $t^{1/2}$ of 66.6 hours for human lymphocytes compared to other human cell lines (Quievryn and Zhitkovich, 2000).

Speit et al. (2000) hypothesized that single peptides or small peptide chains cross-linked to DNA are critical to formaldehyde-induced mutation. However, these authors did not find significant differences in the induction and repair of DPXs in a normal human cell line (MRC4CV1), nucleotide excision repair (NER)-deficient xeroderma pigmentosum (XP) fibroblast cell line, and a Fanconi anemia (FA) cell line exposed to 125–500 μM formaldehyde for 2 hours. In contrast, these cells showed increased susceptibility to formaldehyde-induced MN formation. It is suggested that the

NER pathway affects cytogenetic makers of genotoxicity rather than the cross-link repair (Speit et al., 2000).

DNA Single Strand Breaks (SSBs)

Formaldehyde has been shown to induce SSBs in a number of mammalian cell systems in vitro (see Table A-21). Certain cell lines seem to be more sensitive for SSB formation than others. For example, formaldehyde induced SSBs at concentrations ranging from 0.005−0.8 mM in human primary cells including lung/bronchial epithelial cells (Grafstrom, 1990; Saladino et al., 1985; Grafstrom et al., 1984; Fornace et al., 1982), skin fibroblasts (Snyder and van Houten, 1986; Grafstrom et al., 1984), lymphocytes (LICM, 2006), and in human cell lines A549 (Vock et al., 1999) and HeLa (LICM, 2006) cells, and rat hepatocytes (Demkowicz-Dobrzanski and Castonguay, 1992). In many of these studies SSB induction was dose-dependent. However, formaldehyde did not induce SSBs in human foreskin fibroblasts (Snyder and van Houten, 1986), human skin keratinocytes exposed for 20 hours (Emri et al., 2004), mouse leukemia cells (Ross et al., 1981; Ross and Shipley, 1980) and hamster CHO cells (Marinari et al., 1984) and V79 cells (Speit et al., 2007b).

Formaldehyde induces more DPX than SSBs in normal human bronchial epithelial cells (Grafstrom, 1990; Saladino et al., 1985). Grafstrom et al. (1984) examined the kinetics of DNA repair in nucleotide excision repair (NER)-proficient human bronchial epithelial cells and fibroblasts and NER-deficient fibroblasts from XP patients by alkaline elution technique. They reported comparable levels of DPX in all cell lines, suggesting non-involvement of NER in DPX removal. However, the SSB levels are higher than DPX in XP cells compared to the normal fibroblasts, although both these DNA lesions are repaired at comparable rates, suggesting an additional indirect mechanism of SSB formation possibly involving a different repair pathway. SSBs in HeLa cells induced by 10 µM formaldehyde were repaired by 90 minutes after cells were washed to remove formaldehyde (LICM, 2006).

Cytogenetic markers of genotoxicity

Clastogenic effects, including increased MN, CAs, and SCEs, have been reported in a variety of in vitro systems as shown in Table A-21.

Micronucleus (MN) formation

Studies have shown MN formation either in V79 lung epithelial cell lines (Speit et al., 2007b; Merk and Speit, 1998), in human fibroblasts with varying DNA repair backgrounds (Speit et al., 2000), or in whole blood cultures (Schmid and Speit, 2007). Speit et al. (2000) reported a higher frequency of MN formation in xeroderma pigmentosum (XP) and Fanconi anemia (FA) cell lines compared to normal human cell lines suggesting the importance of NER and crosslink repair following formaldehyde exposure. In V79 cells, Speit et al. (2007b) observed that MN frequency increased with repeated formaldehyde treatments compared to a single treatment; however, such an increase was not observed if the treatment interval was increased to 24 hours. An increase in

micronucleus frequency was observed in mouse erythropoietic cells (Ji et al., 2014), human A549 lung epithelial cells (Speit et al., 2011a), human lymphoblasts (Ren et al., 2013), and human whole blood cultures (Speit et al., 2011a).

Schmid and Speit (2007) observed a statistically significant increase in MN formation at or above a formaldehyde concentration of 300 μM in human whole blood cultures treated with formaldehyde 24 hours after the start of the culture and cytochalasin B (CytB) added 20 hours later (44 hours after the start of the culture). This prompted the conclusion that the level of DPX formation from formaldehyde exposure would need to be high for MN formation and the cells must be exposed after the first mitosis (which is 24 hours). In examining MN formation more closely with Fluorescence In Situ Hybridization (FISH), Schmid and Speit (2007) found that 81 percent of the time, formaldehyde was inducing a micronuclei that was centromere negative indicating the effect to be clastogenic rather than aneugenic (a centromere contained micronuclei).

Sister chromatid exchanges (SCEs)

Sister chromatid exchanges occur as a result of errors in replication process, where an exchange in the chromatids between sister chromatids occurs during the anaphase. DPX are likely to cause replication block and might stimulate SCEs in cells. Therefore, evaluation of SCEs is important in assessing the genotoxicity of formaldehyde.

Formaldehyde has been shown to induce SCEs in most of the in vitro studies, both in rodent and human cells. The available studies are summarized in Table A-21. Different cell types responded differently for various concentrations for formaldehyde, particularly at low doses. For example, the lowest effective concentration (LEC) of formaldehyde in Chinese hamster embryo cells was 0.01 mM, for CHO cells it was 0.03 mM, and V79 cells responded at a concentration of 0.06 mM, while human lymphocytes required slightly higher concentrations (0.125 mM) to show any effect. Neuss and Speit (2008) observed a significant dose-dependent increase in SCE formation in V79 cells and A549 cells following a range of formaldehyde concentrations with 0.1 mM being the LEC when BrdU was added immediately after formaldehyde exposure. However, when BrdU addition was delayed by 4 hours the LEC increased to 0.2 mM suggesting DNA repair. In co-cultivation experiments, the authors first treated A549 cells for 1 hour with 0.05 mM formaldehyde and then co-cultured them with V79 cells with or witout changing the culture medium, SCEs were observed in A549 cells in both situations, but in the co-cultured V79 cells, SCEs were observed only when the medium was not changed, suggesting residual availability of formaldehyde in the medium to induce SCEs in V79 cells and that formaldehyde which entered the A549 cells is either utilized or inactivated. Miyachi and Tsutsui (2005) measured the induction of SCEs in Syrian hamster embryo (SHE) cells at an LEC of 0.01 mM within an hour of formaldehyde exposure. Schmid and Speit (2007) observed that SCEs were induced by 200 μM in lymphocytes from human whole blood cultures, an effect apparently associated with cytotoxicity as indicated by a concomitant reduction in the proliferative index.

Chromosomal aberrations (CAs)

Several studies have demonstrated formaldehyde-induced CAs in a variety of mammalian cells, such as CHO cells (Lorenti Garcia et al., 2009; Natarajan et al., 1983), Chinese hamster lung fibroblasts (Ishidate et al., 1981), Syrian hamster embryo (SHE) cells (Hagiwara et al., 2006; Hikiba et al., 2005), mouse lymphoma cells (Speit and Merk, 2002), human PBLs (Dresp and Bauchinger, 1988; Schmid et al., 1986), and human fibroblasts (Levy et al., 1983).

Hikiba et al. (2005) used SHE cells to measure the induction of CAs following exposure to a series of formaldehyde concentrations (0, 33, 66, and 99 μM) for 24 hours and observed the percentages of aberrant metaphases to be 0, 6, 6, and 71, respectively. The aberrations were predominantly chromosome gaps and chromosomal breaks and exchanges. The relative colony-forming efficiency remained high (at least 85%). Dose-dependent increases in chromosomal aberrations were observed when CHO cells were exposed to 0.15 mM of commercial formaldehyde (Lorenti Garcia et al., 2009). Chinese hamster lung fibroblasts, when exposed to 0.6 mM formalin induced chromosomal aberration within 24 hour of exposure (Ishidate et al., 1981). Note that formalin was used in this study as a source of formaldehyde.

Dresp and Bauchinger (1988) exposed human lymphocytes to various concentrations of formaldehyde. A dose-dependent increase in chromosomal aberrations was observed. Schmid et al. (1986) used the same cell lines and exposed them to 0.25 and 0.5 mM formaldhyde containing 10% methanol. Both chromatid breaks and gaps were observed. It should be recognized that the in vitro studies used different forms of formaldehyde, including commercial grade formaldehyde, paraformaldehyce, formalin (formaldehyde containing 10–15% methanol) or methanol-free formaldehyde.

Mutations and cell transformation

Mutations may occur as a result of the misrepair of formaldehyde-induced DNA damage (DPXs, DNA adducts, SSBs, or clastogenic effects) or as a result of replication errors during mitogenesis. The in vitro evidence for formaldehyde-induced mutations, as discussed below, is strengthened by the correlation between these genotoxic and clastogenic events of formaldehyde and the induction of mutations in other test systems. Numerous studies have demonstrated formaldehyde-induced DNA mutations under a variety of experimental conditions (reviewed in reviewed in IARC, 2012; NTP, 2010; IARC, 2006; Liteplo and Meek, 2003; Conaway et al., 1996; IARC, 1995; Ma and Harris, 1988; Auerbach et al., 1977).

Deletion and point mutations

Several studies demonstrated deletion mutations in cultured mouse lymphoma cells (Speit and Merk, 2002; Mackerer et al., 1996), CHO cells and V79 lung epithelial cells at the hypoxanthine phosphoribosyl transferase (hprt) locus (Merk and Speit, 1999, 1998; Graves et al., 1996; Grafström et al., 1993) as well as in human TK6 lymphoblast cells (Crosby et al., 1988; Craft et al., 1987; Goldmacher and Thilly, 1983) as shown in Table A-21.

Craft et al. (1987) measured the induction of mutations in the thymidine kinase (*tk*) locus or at the ouabain resistance (*Oua*^{r}) locus in TK6 human lymphoblastoid cells. The mutagenesis at *tk* locus can result from base-pair substitutions, small and large deletions, and chromosome exchange events, while mutations at the *Oua*^{r} locus require specific base-pair substitutions. Lymphoblostoid cells were exposed to single (0, 15, 30, 50, 125, or 150 μM for 2 hours) or multiple treatments, that is, 3, 5, or 10 treatments of 50, 30, or 15 μM, respectively, or 4 treatments of 150 μM for 2 hours (treatments were spaced 2–4 days apart) with formaldehyde and mutations analyzed. The authors observed a nonlinear increase in *tk* mutagenesis with single treatment of formaldehyde with increasing slope >125 μM. Although multiple treatments caused an increase in *tk* mutagenesis, their combined effect was less than the single treatment of equivalent C × t (150 μM × 2 hours). No mutations were observed at the *Oua*^{r} locus in lymphoblasts that received four treatments of 150 μM for 2 hours. *Tk* mutagenesis followed a similar exposure-response curve as DPX formation in this study (Craft et al., 1987).

Using the same cell system, Crosby et al. (1988) showed that repetitive treatments of 150 μM formaldehyde induced mutants at the X-linked hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus. Of these mutants, 14/30 of them contained partial or complete deletions with most of the partial deletions showing unique deletion patterns, while only a third (5/15) of spontaneous mutants had partial or complete deletions, indicating that formaldehyde can induce large losses of DNA in human lymphoblast cells. This work was followed up by Liber et al. (1989), who showed that HPRT mRNA from human lymphoblast mutants (16 formaldehyde-induced and 10 spontaneous, both not showing deletions) contained a preferential AT to CG transversion at a specific site (Liber et al., 1989).

Formaldehyde has been shown to induce *hprt* mutations in CHO cells involving single-base pair transversions mostly occurring at AT sequences (Graves et al., 1996). Formaldehyde also induced forward mutations in mouse lymphoma L5178Y tk± cells both in the absence and presence of rat liver S9 (higher concentrations required for effect with S9). Both toxicity and mutagenicity were abolished when formaldehyde dehydrogenase (FADH) was incorporated in the exposure medium (Blackburn et al., 1991), suggesting detoxification of formaldehyde.

A study by Merk and Speit (1998) indicated that formaldehyde-induced DPXs did not result in direct gene mutations in the *hprt* locus of V79 Chinese hamster cells, suggesting that formaldehyde was not mutagenic. However, the *hprt* mutation assay may be insensitive to deletion mutations (Merk and Speit, 1998) because the *hprt* locus in the V79 cell line is primarily sensitive to point mutations. Additionally, one study showed the formation of deletion mutations by formaldehyde at the same locus in human lymphoblasts (Crosby et al., 1988).

In the mouse lymphoma assay (L5178Y cells), Speit and Merk (2002) demonstrated that a 2-hour exposure to formaldehyde was mutagenic in a concentration-dependent manner. Mutation was mainly attributed to a strong increase in small colony mutants suggestive of CAs. Recombination or deletion of DNA from the *tk* locus was primarily responsible for the loss of

heterogeneity, thereby leading to the observed mutant phenotype. This mutagenic finding in the L5178Y cell mouse lymphoma system, which is likely to occur by a clastogenic mechanism rather than by point mutations (Speit and Merk, 2002), is consistent with that of Craft et al. (1987), who demonstrated formaldehyde mutagenicity at the *tk* locus of TK6 cells, and also with the findings of Grafstrom et al. (1984), who demonstrated increased SSB formation in formaldehyde-exposed cell lines.

Transformation

Formaldehyde has also been shown to induce cell transformation in mouse embryo fibroblasts (Boreiko and Ragan, 1983; Frazelle et al., 1983; Ragan and Boreiko, 1981) and hamster kidney cells (Plesner and Hansen, 1983) as shown in Table A-21. In mouse embryonic C3H/10T$^{1/2}$ cells, a single exposure to formaldehyde (0.003−0.083 mM) for 24 hours did not induce transformation; however, when formaldehyde treatment was followed by continuous treatment with 0.1 μg/mL with the tumor promoter 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), a dose-dependent increase in transformation was observed at low concentrations of 0.003 mM (Boreiko and Ragan, 1983) or 0.017 mM (Ragan and Boreiko, 1981) formaldehyde. Ragan and Boreiko (1981) have also shown that treatment of mouse embryo fibroblasts with varying doses of formic acid (≈2 to 22 mM) or methanol (≈0.11 to 1.1 M) did not induce transformation either alone or following TPA promotion in mouse embryo fibroblasts. The authors concluded that since commercial formalin contains 10% methanol, and use of 105 times higher methanol concentrations (≈2.2 M) in this experiment ruled out the background interference of methanol (precursor to formaldehyde) or formic acid (a metabolic product of formaldehyde) with formaldehyde-induced cell transformation. In a different study using the same cells, the ability of formaldehyde to act as a tumor promoter was tested with repeated applications of formaldehyde following initiation with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) by Frazelle et al. (1983) who observed a weak tumor promoting activity of formaldehyde. Another study with a 3-hour exposure to formaldehyde (0.003 to 3.33 mM) with metabolic activation using S9 mix in baby hamster kidney (BHK) cells induced dose-dependent increase in transformation (Plesner and Hansen, 1983).

Expression of p53 mutation and cell death

Four cell lines derived from formaldehyde induced rat nasal squamous cell carcinomas (SCCs) from a previous study (Recio et al., 1992) were analyzed by Bermudez et al. (1994) for p53 mutations as shown in Table A-21. These cell lines were aneuploid overexpressing transforming growth factor-α and epidermal growth factor, expression of which is a common feature of SCCs and is frequently found in human tumors. Two each of these cell lines contained wild type DNA sequences while two others possessed mutated p53 gene sequences, being point mutations, in particular having transversions at codons 132 (TTC→TTA) and 271 (CGT→CAT) of the *p53* gene. In order to understand the mechanism of transformed cell lines conveting to tumor phenotype, the auhors injected either the the wild type or cells with mutant p53 sequnces into nude mice. They

observed that only cell lines expressing the p53 mutation were tumorigenic, suggesting involvement of specific p53 mutations in the tumorigenicity of formaldehyde. Wong et al. (2012) examined signal transduction pathways in response to formaldehyde exposure. The authors studied p53 phosphorylation in human lung epithelial (H460 cells) and fibroblast cells exposed to formaldehyde and compared the role of different protein kinases using specific inhibitors for ATR, ATM, and DNA, measuring Ser15p53 and thr68-CHK1 phosphorylation, p53 accumulation, and induction of p21. At low doses, formaldehyde-induced DNA-protein crosslinks caused ATR-mediated activation of p53 in human lung fibroblasts and epithelial cells. The S-phase of the cell cycle seems to be specifically sensitive for this effect without the involvement of topoisomerase binding protein 1 (topBP1). Other pathways, such as BER and NER, mismatch repairs were not affected by p53 activation, suggesting that non-DPX adducts, including DNA-peptide and hmDNa adducts, play a minor role in formaldehyde-induced p53 activation.

Other genotoxic endpoints

As summarized in Table A-21, in vitro formaldehyde exposure induces other genotoxic and related effects in mammalian cells such as UDS and DNA repair inhibition.

Unscheduled DNA synthesis

UDS, which represents DNA repair activity following excision of DNA damage, has been reported in rat hepatocytes (Williams et al., 1989b) and SHE cells (Hamaguchi and Tsutui, 2000) exposed to formaldehyde. UDS was also observed in HeLa cells (Martin et al., 1978), but not in human bronchial epithelial cells (Doolittle et al., 1985) upon formaldehyde exposure. These studies suggest that formaldehyde-induced DNA damage was followed by DNA repair.

DNA repair inhibition

Formaldehyde can inhibit DNA repair and induce cell transformation (Emri et al., 2004; Speit et al., 2000; Grafstrom et al., 1984; Boreiko and Ragan, 1983) as shown in Table A-21. Studies have shown that formaldehyde causes DNA repair inhibition at a concentration range of 0.125 mM to 10 mM in human bronchial epithelial cells (Grafstrom et al., 1984) and skin fibroblasts or keratinocytes (Emri et al., 2004), DNA repair proficient or deficient cell lines (e.g., XP), or cell lines hypersensitive to DNA-DNA crosslinks (e.g., FA) (Speit et al., 2000). In a study using human keratinocytes and fibroblasts, Emri et al. (2004) tested the formation of DNA SSBs induced by ultraviolet (UV) irradiation by UVB or UVC with or without prior treatment with 10 μM formaldehyde. The authors reported that SSB induced by UV irradiation alone were repaired within 3–6 hours of exposure, while cells with UV irradiation followed by formaldehyde exposure had higher SSBs at the same time points due to increased chromosomal damage, suggesting that formaldehyde exposure altered the repair kinetics in these cells.

Aneuploidy

Studies on aneuploidy in various in vitro and human cell systems have provided mixed results as shown in Table A-21. For example, increase in aneuploidy was observed in hamster CHO cells (Kumari et al., 2012) and human erythropoietic stem cells (Ji et al., 2014). However, no increase in aneuploidy cells were observed in hamster V79 lung epithelial cells (Kuehner et al., 2012; Speit et al., 2011a) or in human myeloid progenitor cells (Kuehner et al., 2012).

Table A-21. Summary of in vitro genotoxicity studies of formaldehyde in mammalian cells

Test system	Dose/ Concentration[a]	Results[b] -S9	Results[b] +S9	Comments (duration; endpoint method; toxicity)	Reference
p53 Mutations					
Rat Nasal tumor cell lines	NA	+	ND	cell lines derived from nasal tumors of rats from 2-yr tumor study; rats exposed to 18.5 mg/m^3 HCHO, 6 hrs/d, 5 d/wk for 2 yrs	(Bermudez et al., 1994)
Deletion mutations					
Mouse Lymphoma L5178Y tk$^{+/-}$ cells	0.063 mM HCHO (commercial)	+	ND	2 hrs; mouse lymphoma assay; cytotoxic at 250 μM conc.	(Speit and Merk, 2002)
	0.8 mM 37% HCHO + 10% methanol	ND	+	3 hrs; MF at TK locus; 40–50% total growth at 0.8 mM dose	(Mackerer et al., 1996)
Hamster CHO cells/*Hprt* locus	0.3 mM HCHO (37% w/w)	+	ND	1 hr; 6-TG resistant mutants; dose-dependent ↓ in CFE and ↑ in MF	(Grafström et al., 1993)
	0.5 mM HCHO (commercial)	–	ND	4 hrs; HPRT assay; (T) by relative CE ≥ 0.125 mM	(Merk and Speit, 1998)
	1 mM HCHO (40% aq. Sol.)	+	ND	1 hr; 6-TG resistant colonies; base transversions at AT base pairs	(Graves et al., 1996)
Hamster V79 lung epithelial cells	0.5 mM HCHO (commercial)	–	ND	4 hrs; HPRT assay; (T) by relative CE ≥ 0.25 mM	(Merk and Speit, 1999)
Human Bronchial fibroblasts/epithelial cells (*HPRT* locus)	0.1 mM HCHO (commercial)	+	ND	5 hrs; 6-TG resistant mutants scored; MF nonlinear dose-dependent ↑; (T) > 0.1 mM by CFE	(Grafstrom et al., 1985)
Human Lymphoblast/TK6	0.03 mM 37% HCHO + 10-15% methanol	+	ND	2 hrs; MF at TK locus measured; single exposure (0–150 μm) nonlinear ↑ in MF; (T) at 0.125 mM	(Craft et al., 1987)
	0.13 mM 37% HCHO + 10-15% methanol	+	ND	2 hrs; MF at TK locus; cell survival was 15% at 0.15 mM; cells treated for 2 hrs with	(Goldmacher and Thilly, 1983)

Test system	Dose/ Concentration[a]	Results[b] -S9	Results[b] +S9	Comments (duration; endpoint method; toxicity)	Reference
				0.07 mM methanol were not mutagenic, not cytotoxic	
	0.15 mM HCHO (commercial)	+	ND	8 exposures × 4 d, 2 hrs dosing; MF at HPRT locus; MF 12.4-fold higher over background; (T) 50% survival each treatment	(Crosby et al., 1988)
Point mutations					
Mouse Lymphoma cell/ TK+/-	0.1 mM (-S9) and 0.5mM (+S9) 37% HCHO +10% methanol	+,–	+,–	NR; assay supplemented with FDH and NAD+; MF at the TK locus; results indicate without and with FDH/NAD^+, respectively; 50% (T) at 0.1 mM (-S9) and 0.5 mM (+S9) with FDH	(Blackburn et al., 1991)
	0.14 mM HCHO form not specified	+	ND	4 hrs; MF at TK locus; highly mutagenic but total growth is very low	(Wangenheim and Bolcsfoldi, 1988)
Hamster CHO cells/*Hprt* locus	1 mM HCHO (40% aq. Sol.)	+	ND	1 hr; 6-TG resistant colonies had base transversions at AT base pairs	(Graves et al., 1996)
Human Lymphoblast/TK6	0.15 mM HCHO (commercial)	+	ND	2 hrs (8 times); sequence analysis of HPRT mutants showed base substitutions at AT base pairs	(Liber et al., 1989)
DNA-protein crosslinks					
Mouse Hepatocytes	0.5 mM [^{14}C] HCHO (aq. Sol.)	+	ND	2 hrs; nonlinear dose-dependent ↑ in DPX.	(Casanova et al., 1997)
	0.5 mM [^{14}C] HCHO (aq. Sol.)	+	ND	2 hrs; HPLC analysis of DNA digest; Dose-dependent ↑ in DPX.	(Casanova and Heck, 1997)
Mouse L5178Y $tk^{+/-}$ Lymphoma cells	0.031 mM HCHO (commercial)	+	ND	2 hrs; DPX show dose-response; cytotoxic at 250 μM conc.	(Speit and Merk, 2002)
Mouse Leukemia L1210 cells	0.125 mM 37% HCHO	+	ND	1 hr; (T) at 0.3 μM conc.	(Ross et al., 1981)
	0.2 mM 37% HCHO	+	ND	2.5 hrs; (T) ≥ 0.175 mM	(Ross and Shipley, 1980)
Mouse Bone marrow mesenchymal cells	0.125 mM HCHO (37%)	+	ND	12 hrs; Alkaline comet assay; (T) from 0.175 mM to 0.2 mM	(She et al., 2013)
Rat C18 tracheal epithelial cell line	0.1 mM PFA in PBS	+	ND	1.5 hrs; DPX analyzed by alkaline elution; (T) at 0.4 mM	(Cosma and Marchok, 1988)

Test system	Dose/ Concentration[a]	Results[b]		Comments (duration; endpoint method; toxicity)	Reference
		-S9	+S9		
Rat Aortic endothelial cells	0.5 mM HCHO (commercial)	+	ND	1.5 hrs; K+/SDS assay; dose-dependent ↑ in DPX ≥ 2 hrs; (T) by LDH release at 2 mM	(Lin et al., 2005)
Rat Primary tracheal epithelial cells	0.05 mM PFA in PBS	+	ND	1.5 hrs; DPX analyzed by alkaline elution; (T) > 0.2 mM	(Cosma and Marchok, 1988)
	3.34 mM HCHO/PBS	+	ND	3 hrs; dose-dependent ↑ in DPX	(Cosma and Marchok, 1988)
Rat Yoshida lymphosarcoma cells	0.25 mM HCHO (36% sol)	+	ND	4 hrs; alkaline elution assay; (T) ID_{50} 0.25 mM	(O'Connor and Fox, 1987)
Hamster CHO cells	0.125 mM HCHO (commercial)	+	ND	2 hrs; BrdU incorporation-FPG technique; conc.-related ↓ DNA migration inhibition;	(Lorenti Garcia et al., 2009)
	0.2 mM HCHO (NS)	+	ND	1.5 hrs; dose-dependent ↑ in DPX up to 2 mM HCHO; values visually determined from graph	(Zhitkovich and Costa, 1992)
	0.25 mM HCHO (NS)	+	ND	1.5 hrs; dose-dependent ↑ in DPX formation up to 2 mM HCHO; values visually determined from graph	(Olin et al., 1996)
	0.5 mM HCHO (commercial)	+	ND	1.5 hrs; alkaline elution assay; DPX showed dose-dependent ↑(0.5–4.5 mM); 82% viability at 4.5 mM HCHO	(Marinari et al., 1984)
Hamster V79 lung epithelial cells	0.01 mM 16% HCHO (ultrapure methanol free)	+	ND	1 hr; Comet assay; dose-dependent ↓ in DNA migration at HCHO ≥ 0.01 mM;	(Speit et al., 2007b)
	0.025 mM 16% HCHO (ultrapure methanol free);	+	ND	4 hrs; Comet assay; dose-dependent ↓ DNA migration; (T) at 0.2 mM by cell counts/proliferation index;	(Speit et al., 2008a)
	0.0625 mM HCHO (commercial)	+	ND	4 hrs; Comet assay; dose-dependent ↑ migration inhibition (0.0625–0.5 mM); (T) by relative CE ≥ 0.25 mM;	(Merk and Speit, 1999)
	0.125 mM HCHO (commercial)	+	ND	4 hrs; K-SDS assay; nonlinear dose-dependent ↑ in DPX (values visually determined from graph); HCHO (T) by relative CE assay ≥ 0.125;	(Merk and Speit, 1998)
Human Nasal epithelial cells	0.2 mM 16% HCHO (ultrapure methanol free)	+	ND	1 hr; Comet assay; dose-dependent ↑ DPX from 0.05–0.3 mM; (T) by CF ≥ 0.02 mM;	(Speit et al., 2008b)

Test system	Dose/ Concentration[a]	Results[b] -S9	Results[b] +S9	Comments (duration; endpoint method; toxicity)	Reference
Human A549 lung epithelial cells	0.2 mM 16% HCHO (ultrapure Methanol free)	+	ND	1 hr & 4 hrs; Comet assay; dose-dependent ↑ migration inhibition from 0.1–0.3 mM; (T) by CF ≥ 0.02 mM;	(Speit et al., 2008b)
	0.2 mM HCHO (stabilized with Methanol)	+	ND	3 hrs; KCl/SDS method; DPX time-dependent ↑ up to 12 hrs; $T^1/_2$ 12.5 hrs; (T) ≥ 0.2 mM by CF assay,	(Quievryn and Zhitkovich, 2000)
	0.2 mM 16% HCHO aq. sol., methanol-free	+	ND	1 or 3 x 24 hr intervals; comet assay	(NTP, 2010)
Human Lung/bronchial epithelial cells	0.1 mM HCHO (commercial)	+	ND	1 hr; alkaline elution technique; (T) 0.021 mM ID_{50} by growth inhibition	(Saladino et al., 1985)
	0.1 mM HCHO (commercial)	+	ND	1 hr; alkaline elution technique; (T) at 0.3 mM by CFE	(Grafstrom et al., 1986)
	0.2 mM 37% HCHO (w/w)	+	ND	1 hr; alkaline elution technique; (T) at 1 mM	(Grafstrom et al., 1984)
	2 mM HCHO (Not Specified)	+	ND	1 hr; Alkaline elusion technique;	(Grafstrom, 1990)
	0.39 mM HCHO	+	ND	4 hrs; KCl-SDS method	(Duan, 2011)
	0.8 mM 37% HCHO	+	ND	1 hr; alkaline elution;	(Fornace et al., 1982)
Human Bronchial epithelial cells/fibroblasts	0.1 mM 37% HCHO	+	ND	1 hr; alkaline elution technique;	(Grafstrom et al., 1983)
Human Fibroblasts (diploid)/HF/SV40	0.2 mM HCHO + Methanol)	+	ND	3 hrs; (T) ≥ 0.2 mM by CF assay; DPX half life is 12.5 hrs	(Quievryn and Zhitkovich, 2000)
Human Fibroblast (Bronchial/Skin)	0.25 mM HCHO (NS)	+	ND	1.5 hrs; DPX dose-response not prominent; values visually determined from graph	(Olin et al., 1996)
Human Skin keratinocytes/ fibroblasts	0.025 mM HCHO (NS)	+	ND	8 hrs with subsequent exposure to methyl methane sulfonate (0.25 mM)	(Emri et al., 2004)
Human XP fibroblasts	0.2 mM 37% HCHO (w/w)	+	ND	1 hr; alkaline elution technique; DPX $T^1/_2$ 2-3 hrs	(Grafstrom et al., 1984)
Human Normal, XPA and FA repair deficient fibroblasts	0.125 mM HCHO (commercial)	+	ND	2 hrs; Comet assay; dose-dependent DNA migration inhibition; No migration inhibition after 24 hrs;	(Speit et al., 2000)
Human Fibroblasts/XP-F and	0.2 mM HCHO (stabilized with	+	ND	3 hrs; DPX removal XP-A = XP-F cells; (T) ≥ 0.2 mM by CF	(Quievryn and

Test system	Dose/ Concentration[a]	Results[b] -S9	Results[b] +S9	Comments (duration; endpoint method; toxicity)	Reference
XP-A	Methanol)			assay;	Zhitkovich, 2000)
Human Lymphocytes	0.05 mM 10% formalin	+	ND	1 hr; comet assay; KCl/SDS assay; nonlinear dose-dependent ↑ ≥ 50 μM HCHO	(LICM, 2006)
	0.1 mM; 0.3 mM HCHO in water	+	-	3 hrs; (T) at 0.3 mM (+S9)	(Andersson et al., 2003)
	0.2 mM HCHO + Methanol)	+	ND	3 hrs; KCl/SDS method; DPX $T^1/_2$ 18.1 hrs; (T) ≥ 0.2 mM by CF assay,	(Quievryn and Zhitkovich, 2000)
Human White blood cells	0.001 mM HCHO (NS)	+	ND	1.5 hrs; Dose-dependent ↑ in DPX formation up to 2 mM HCHO; values visually determined from graph	(Shaham et al., 1996)
Human Whole blood cultures	0.025 mM 16% HCHO (ultrapure Methanol free)	+	ND	exposure duration not specified; Comet assay; dose-dependent migration inhibition; DPX ≥ 0.2 mM persist for 24 hrs;	(Schmid and Speit, 2007)
Human Lymphoblast/TK6	0.05 mM 37% HCHO + 10-15% Methanol	+	ND	2 hrs; MF at TK locus measured; (T) at 0.125 mM	(Craft et al., 1987)
Human Lymphoblast/TK6	0.1 mM 16% HCHO (ultrapure MetOH free)	+	ND	2 hrs; Comet assay with g-irradiation; DPX formation dose-dependent; (T) at 0.1 mM 24 hrs by MTT assay	(Kuehner et al., 2013)
Human lymphoblasts (PD20 & PD20-D2)	0.125 mM 37% HCHO	+	ND	24 hrs; Dose-dependent ↑ in DPX from 0.05-0.15 mM; PD20>PD20-D2; (T) >0.15 mM	(Ren et al., 2013)
Human EBV-Burkitt's lymphoma cells	0.03% PFA in water	+	ND	18 hrs; Dose-dependent ↑ in DPX; (T) 0.01% PFA	(Costa et al., 1997)
Human T-leukemia (Jurkat E6-1) cells	1 mM HCHO (commercial)	+	ND	2 hrs; SDS-PAGE; (T) ≥ 1 mM by cell death assay	(Saito et al., 2005)
Human HeLa cells	0.05 mM 10% formalin	+	ND	1 hr; KCl/SDS precipitation method; (T) ≥ 100 mM by absorbance after 12 hrs; dose-dependent ↑ in DPX; repaired within 18 hrs after HCHO removal	(LICM, 2006)
Human Kidney cells/Ad293	0.2 mM HCHO + Methanol	+	ND	3 hrs; KCl/SDS method; DPX $T^1/_2$ 12.5 hrs; (T) ≥ 0.2 mM by CF assay,	(Quievryn and Zhitkovich, 2000)
Human Gastric mucosa cells	1 mM HCHO	+	ND	1 hr; (T) not reported	(Blasiak et al.,

Test system	Dose/ Concentration[a]	Results[b] -S9	Results[b] +S9	Comments (duration; endpoint method; toxicity)	Reference
					2000)
DNA adducts					
Hamster CHO cells	1 mM [^{3}H] 37% HCHO/10-15% Methanol	+	ND	2 hrs; (T) ≥ 2.5 mM	(Beland et al., 1984)
Human Nasal epithelial cells	0.33 mM 37% HCHO + 10% Methanol	+	ND	24 hrs; hmdA and hmdG adducts dose-dependent ↑ . Viability showed dose-dependent from 10 500 mM;	(Zhong and Que Hee, 2004)
Human HeLa cells	0.5 mM [$^{13}CD_2$]HCHO (20% in heavy water)	+	ND	3 hrs; No (T) information provided.	(Lu et al., 2012a)
Chromosomal aberrations (CA)					
Hamster CHO cells (AA8) and their mutants (UV4, UV5, UV61)	0.15 mM HCHO (commercial)	+	ND	2 hrs; BrdU incorporation-FPG technique; dose-dependent ↑ in Cas	(Lorenti Garcia et al., 2009)
Hamster CHO cells	0.2 mM PFA in water	+	+	2 hrs; BrdU incorporation; dose-dependent ↑ in SCE +/- S9;	(Natarajan et al., 1983)
Hamster CHO cells mutants (KO40)	0.2 mM HCHO (commercial)	+	ND	2 hrs; BrdU incorporation-FPG technique; dose-dependent ↑ in CAs	(Lorenti Garcia et al., 2009)
Hamster CHO cells	0.53 mM HCHO	(+)	(+)	8–12 hrs; Giemsa staining;	(Galloway et al., 1985)
Hamster Lung fibroblasts	0.6 mM Formalin	+	ND	24 hrs; microscopic evaluation	(Ishidate et al., 1981)
Hamster/Syrian Embryo cells	0.033 mM 37% HCHO + 7–13% Methanol	+	ND	24 hrs; CA assay; 85% relative CFE at 0.099 mM	(Hikiba et al., 2005)
Human Fibroblasts	2 mM HCHO (NS)	+	ND	0.25 hr; Giemsa staining; dose-dependent ↑ in CA;	(Levy et al., 1983)
Human Lymphocytes	0.125 mM HCHO (NS)	+	ND	1 hr; PCC technique; dose-dependent↑ in CA	(Dresp and Bauchinger, 1988)
Human lymphoblasts (PD20 & PD20-D2)	0.125 mM 37% HCHO	+	ND	24 hrs; Dose-dependent ↑ in CA from 0.05-0.15 mM; PD20=PD20-D2; (T) >0.15 mM	(Ren et al., 2013)
Human lymphocytes	0.25 mM, 0.5 M 37% HCHO + 10% Methanol	+	+	1 hr; conc. Respectively, for chromatid breaks and gaps; proliferation inhibition at 1 M (-S9) and 0.5 mM (+S9)	(Schmid et al., 1986)
Micronucleus (MN)					

Test system	Dose/ Concentration[a]	Results[b] -S9	Results[b] +S9	Comments (duration; endpoint method; toxicity)	Reference
Mouse erythropoietic cells	0.025 mM HCHO (37% + 10-15% methanol)	+	ND	1 hr; Dose-dependent in MN from 0.025-0.1 mM;	(Ji et al., 2014)
Hamster V79 lung epithelial cells	0.075 mM 16% HCHO (ultrapure Methanol free);	+	ND	2 hrs; MN test; MN ≥ 0.075 mM; dose-dependent ↑ in MN;	(Speit et al., 2007b)
	0.1 mM 16% HCHO (ultrapure Methanol-free);	+	ND	4 hrs; MN test; dose-dependent in MN; (T) at 0.2 mM by cell counts/proliferation index;	(Speit et al., 2007b)
	0.125 mM HCHO (commercial)	+	ND	4 hrs; MN assay with AO staining; nonlinear dose-dependent ↑ in MN (values visually determined from graph); (T) by relative CE ≥ 0.125 mM;	(Merk and Speit, 1998)
Human A549 lung epithelial cells	0.15 mM 16% HCHO (ultrapure, methanol-free)	+	ND	2 hrs (0.3 mM) or 30 hrs (0.15 mM); CBMN assay; Mostly centromere -ve by FISH analysis	(Speit et al., 2011a)
Human Normal, XPA and FA repair deficient fibroblasts	0.125 mM HCHO (commercial)	+	ND	2 hrs; MN test; MN ≥ 0.075 mM; dose-dependent ↑ in MN; normal<XPA<FA;	(Speit et al., 2000)
Human lymphoblasts (PD20 & PD20-D2)	0.125 mM 37% HCHO	+	ND	24 hrs; Dose-dependent ↑ in MN from 0.05-0.15 mM;PD20>PD20-D2; (T) >0.15 mM	(Ren et al., 2013)
Human Whole blood cultures	0.3 mM 16% HCHO (ultrapure, methanol-free)	+	ND	27 hrs; CBMN assay; mostly centromere negative by FISH analysis	(Speit et al., 2011a)
Human Whole blood cultures	0.3 mM 16% HCHO (ultrapure Methanol free);	+	ND	24 hrs; HCHO dosed 44 hrs after culture; MN test; dose-dependent ↑ in MN (0.1–0.4 mM); (T) ≥ 0.3 mM by NDI;	(Schmid and Speit, 2007)
Single strand breaks (SSB)					
Mouse Leukemia L1210 cells	0.125 mM 37% HCHO	-	ND	1 hr; (T) at 0.3 mM	(Ross et al., 1981)
	0.2 mM 37% HCHO	(+)	ND	2.5 hrs; (T) ≥ 0.175 mM	(Ross and Shipley, 1980)
Rat Hepatocytes	1 mM HCHO (NS)	+	ND	4 hrs; HCHO cytotoxic ≥1.5 mM; dose-dependent ↑ in SSB, enhanced by GSH depletion	(Demkowicz-Dobrzanski and Castonguay, 1992)
Rat -tracheal epithelial cell line	0.2 mM PFA in PBS	+	ND	1.5 hrs; SSB analyzed by alkaline elution; HCHO toxic at	(Cosma and

Test system	Dose/ Concentration[a]	Results[b] -S9	Results[b] +S9	Comments (duration; endpoint method; toxicity)	Reference
				0.4 mM	Marchok, 1988)
Rat Yoshida lymphosarcoma cells	0.25 mM HCHO (36% sol)	+	ND	4 hrs; alkaline elution assay; (T) ID_{50} 0.25 mM	(O'Connor and Fox, 1987)
Hamster CHO cells	4.5 mM HCHO (commercial)	–	ND	1.5 hrs; 82% viability at 4.5 mM HCHO	(Marinari et al., 1984)
Hamster V79 lung epithelial cells	0.2 mM 16% HCHO (ultrapure Methanol free)	–	ND	1 hr; Comet assay;	(Speit et al., 2007b)
Human Bronchial epithelial cell	0.1 mM 37% HCHO	+	ND	1 hr; alkaline elution technique; (T) at 0.3 mM	(Grafstrom et al., 1983)
	0.3 mM 37% HCHO (w/w)	+	ND	1 hr; SSB dose-dependent ↑; SSB 3 times higher than XP cells	(Grafstrom et al., 1984)
Human Lung/bronchial epithelial cells	0.1 mM HCHO (commercial)	+	ND	1 hr; alkaline elution technique; (T) 0.021 mM ID_{50} by growth inhibition	(Saladino et al., 1985)
	0.1 mM HCHO (commercial)	+	ND	1 hr; alkaline elution technique; (T) at 0.3 mM by CFE	(Grafstrom et al., 1986)
	0.8 mM 37% HCHO	+	ND	1 hr; alkaline elution;	(Fornace, 1982)
Human Lung/bronchial epithelial (A549) cells	1.0 mM HCHO (commercial)	+	ND	8–72 hrs; Dose-dependent in ↑ DSB formation; DSB formed when viability, determined by MTT assay, was >60%	(Vock et al., 1999)
Human Skin keratinocytes/ fibroblasts	0.1 mM HCHO (NS)	–	ND	20 hrs	(Emri et al., 2004)
Human XP fibroblasts	0.3 mM 37% HCHO (w/w)	+	ND	1 hr; SSB dose-dependent ↑	(Grafstrom et al., 1984)
Human Foreskin fibroblasts	0.1 mM 37% HCHO + 10% Methanol	+	ND	0.5 hr; nick translation assay; low doses induce SSB	(Snyder and van Houten, 1986)
	0.25 mM 37% HCHO + 10% Methanol	–	ND	0.5 hr; alkaline sucrose sedimentation analysis; high doses don't induce SSB	(Snyder and van Houten, 1986)
Human HeLa cells	0.005 mM 10% formalin	+	ND	1 hr; Comet assay; (T) ≥ 100 μM after 12 hrs; SSB repaired within 90 min	(LICM, 2006)
Human Lymphocyte, peripheral blood	0.005 mM 10% formalin	+	ND	1 hr; comet assay; KCl/SDS assay; nonlinear dose-dependent ↑ ≥ 50 μM HCHO	(LICM, 2006)
Sister chromatid exchanges (SCE)					

Test system	Dose/ Concentration[a]	Results[b]		Comments (duration; endpoint method; toxicity)	Reference
		-S9	+S9		
Hamster CHO cells	0.03 mM 37% HCHO with 10% methanol	+	ND	24 hrs; BrdU incorporation; SCE dose-dependent ↑	(Obe and Beek, 1979)
	0.04 mM HCHO (commercial)	(+)	(+)	26 hrs; BrdU incorporation-FPG technique	(Galloway et al., 1985)
	0.2 mM PFA in water	+	+	2 hrs; BrdU incorporation; dose-dependent ↑ in SCE +/− S9;	(Natarajan et al., 1983)
Hamster CHO cells (AA8) and their mutants (UV4, UV5, UV61, KO40)	0.15 mM HCHO (commercial)	+	ND	2 hrs; BrdU incorporation-FPG technique; dose-dependent ↑ in CAs	(Lorenti Garcia et al., 2009)
Hamster Embryo cells	0.01 mM 37% HCHO/7−13% Methanol;	+	ND	24 hrs; BrdU incorporation; dose-dependent ↑ in SCE; (T) by relative CE 68% at 0.033 mM	(Miyachi and Tsutsui, 2005)
Hamster V79 lung epithelial cells	0.05 mM 16% HCHO (ultrapure, methanol-free)	+	ND	24 or 28 hrs exposure to HCHO and BrdU; Aneuploidy and Toxicity measured by SCE and PI, respectively.	(Speit et al., 2011a)
	0.06 mM 37% HCHO with 10% methanol	+	–	28 hrs; formalin + activation with primary rat hepatocytes; (T) at 0.54 mM (+S9) and 0.2 mM (−S9)	(Basler et al., 1985)
	0.1 mM 16% HCHO (ultrapure Methanol free);	+	ND	2 hrs; BrdU labeling; SCE ≥ 0.1 mM; genotoxicity paralleled cytotoxicity; (T) ≥ 0.1 mM by PI	(Speit et al., 2007b)
	0.1 mM 16% HCHO (ultrapure Methanol free);	+	ND	1 hr; BrdU labeling; SCE dose-dependent ↑(0.1-0.2 mM)	(Neuss and Speit, 2008)
	0.1 mM 16% HCHO (ultrapure Methanol free);	+	ND	4 hrs; BrdU labeling; dose-dependent in SCE; (T) at 0.2 mM by cell counts/proliferation index;	(Speit et al., 2008a)
	0.125 mM HCHO (commercial)	+	ND	4 hrs; BrdU incorporation; dose-dependent ↑ in SCE; (T) by relative CE ≥ 0.125 mM	(Merk and Speit, 1998)
	0.125 mM HCHO (commercial)	+	ND	4 hrs; BrdU incorporation; dose-dependent ↑ in SCE; (T) by relative CE ≥ 0.25 mM	(Merk and Speit, 1999)
	0.13 mM 37% HCHO with 10% methanol	+	ND	2 hrs; (T) at 0.54 mM	(Basler et al., 1985)
	0.13 mM; 0.20 mM 37% HCHO with	+	–	3 hrs; (T) at 0.4 mM (−S9)	(Basler et al., 1985)

Test system	Dose/ Concentration[a]	Results[b] -S9	Results[b] +S9	Comments (duration; endpoint method; toxicity)	Reference
	10% methanol				
Human A549 lung epithelial cells	0.1 mM 16% HCHO (ultrapure Methanol free);	+	ND	1 hr; BrdU labeling; SCE dose-dependent ↑ (0.1–0.3 mM)	(Neuss and Speit, 2008)
Human A549 + V79 (co-cultivated)	0.05 mM 16% HCHO (ultrapure Methanol free);	+	ND	1 hr; BrdU labeling; SCE dose-dependent ↑ (0.05–0.2 mM); treated A549 cells not washed before adding V79 cells	(Neuss and Speit, 2008)
Human A549 + V79 (co-cultivated)	0.3 mM 16% HCHO (ultrapure Methanol free);	–	ND	1 hr; BrdU labeling; treated A549 cells washed before adding V79 cells	(Neuss and Speit, 2008)
Human Lymphocytes	0.125 mM 37% HCHO + 10% Methanol	+	+	1 hr; BrdU labeling; proliferation inhibition at 1 M (-S9) and 0.5 mM (+S9)	(Schmid et al., 1986)
	0.167 mM 37% HCHO + 10% Methanol	+	ND	24 hrs; BrdU incorporation; dose-dependent ↑ in SCE	(Obe and Beek, 1979)
	0.167 mM formalin or PFA	+	ND	72 hrs; BrdU incorporation with fluorescence + Giemsa method; (T) ≥0.33 mM and similar for formalin and PFA; dose-dependent ↑ for formalin reported	(Krieger et al., 1983)
Human Whole blood cultures	0.2 mM 16% HCHO (ultrapure Methanol free)	+	ND	72 hrs; BrdU labeling; no dose-response; (T) at 0.2 mM by PI	(Schmid and Speit, 2007)
Unscheduled DNA synthesis (UDS)					
Rat Hepatocytes	400 mM HCHO (NS)	+	ND	18–20 hrs; [^{3}H]dThd incorporation and autoradiography	(Williams et al., 1989a)
Human Bronchial epithelial cells	0.1 mM 37% HCHO (reagent grade sol.)	–	ND	22 hrs; [^{3}H]dThd incorporation and autoradiography; (T) ≥ 1 mM	(Doolittle et al., 1985)
Human Foreskin fibroblasts	0.5 mM 37% HCHO + 10% Methanol	–	ND	0.5 hr; UDS	(Snyder and van Houten, 1986)
Human Bronchial fibroblasts	1 mM 37% HCHO	–	ND	1 hr; [^{3}H-Thymidine] incorporation.	(Grafstrom et al., 1983)
Human Embryo cells	0.1 mM HCHO (37% sol)	+	ND	1 hr; [^{3}H]dThd incorporation; dose-dependent ↑ in UDS (0.1-1 mM)	(Hamaguchi and Tsutui, 2000)
Human HeLa cells	0.001 mM HCHO (commercial)	+	ND	2.5 hrs; [^{3}H]dThd incorporation	(Martin et al., 1978)
DNA repair inhibition					

Test system	Dose/ Concentration[a]	Results[b] -S9	Results[b] +S9	Comments (duration; endpoint method; toxicity)	Reference
Human Skin keratinocytes/fibroblasts	0.01 mM HCHO (NS)	+	ND	0.5 hr after exposure to UVB	(Emri et al., 2004)
Human Normal, XPA and FA repair deficient fibroblasts	0.125 mM HCHO (commercial)	+	ND	2 hrs	(Speit et al., 2000)
Cell transformation					
Mouse Embryo fibroblast/C3H10T$^1/_2$ cells	0.003 mM HCHO (37%)	+	ND	24 hrs; HCHO treatment followed by TPA treatment, transformation +ve and dose-dependent; (T) ≥ 0.017 mM	(Boreiko and Ragan, 1983)
	0.017 mM HCHO (37% w/w) exposure	+	ND	24 hrs HCHO, 6 wks to medium ± TPA. HCHO +TPA +ve, dose-dependent ↑ (0.017-0.34 mM); HCHO alone −ve (0.083 mM); methano + TPA or formic acid + TPA −ve. HCHO cytotoxic at 0.033 mM	(Ragan and Boreiko, 1981)
Mouse Embryo fibroblast/C3H10T$^1/_2$ cells	0.033 mM HCHO (37% w/w) exposure;	[+]	ND	4 hrs initiation with 0.5 μg/mL MNNG, promotion on days 5, 8, 15, 22, 29, 36 with HCHO with change of medium	(Frazelle et al., 1983)
Hamster Kidney cell/BHK-21/cI.13	0.03 mM HCHO 37% aq.sol.	+	+	3 hrs; Style's cell transformation assay; transformation dose-dependent ↑ (0.03-0.67 mM); (T) ≥ 0.67 mM	(Plesner and Hansen, 1983)
Aneuploidy					
Hamster CHO cells (WT & XPF-deficient)	0.3 mM HCHO (Not Specified)	+	ND	4 hrs; Wright's stain and G-banding; +ve for tetraploidies and polyploidies	(Kumari et al., 2012)
Hamster V79 lung epithelial cells	0.05 mM HCHO, 16% ultra-pure, methanol-free	−	ND	7 d exposure; FISH analysis; (T) at 0.05 mM by CFA	(Kuehner et al., 2012)
Hamster V79 lung epithelial cells	0.1 mM HCHO, 16% ultra-pure, methanol-free	−	ND	24 or 28 hrs exposure to HCHO and BrdU; Aneuploidy and Toxicity measured by SCE and PI, respectively.	(Speit et al., 2011a)
Human A549 lung epithelial cells	0.05 mM HCHO, 16% ultra-pure, methanol-free	−	ND	14 d exposure; FISH analysis; (T) at 0.02 mM by CFA	(Kuehner et al., 2012)
Human myeloid progenitor cells	0.05 mM HCHO, 16% ultra-pure, methanol-free	−	ND	9 d exposure; Aneuploidy in chromosomes 6, 7, and 8 tested by FISH analysis; (T) at	(Kuehner et al., 2012)

Test system	Dose/ Concentration[a]	Results[b] -S9	Results[b] +S9	Comments (duration; endpoint method; toxicity)	Reference
				0.1 mM by CFA	
Human erythropoietic stem cells	0.05 mM HCHO (37% +10–15% methanol)	+	ND	5 d; FISH analysis; Combined analysis of monosomies or trisomies of 7 and 8 are positive.	(Ji et al., 2014)

[a]Lowest effective concentration (LEC) for positive results or highest ineffective concentration tested (HIC) for negative or equivocal results.

[b]+ = positive; – = negative; (+), equivocal.

6-TG, 6-thioguanine; CF, colony formation; FA, Fanconi anemia; FDH, formaldehyde dehydrogenase; FPG, fluorescence plus Giemsa technique; HCHO, formaldehyde; hmdA, hydroxymethyl-deoxyadenosine; hmdG, hydroxymethyl-deoxyguanosine; hmDNA, hydroxymethyl-DNA; HPRT, hypoxanthine phosphoribosyl transferase; ID_{50}, HCHO concentration causing 50% growth inhibition compared to control cells; MF, mutation frequency; MN, micronucleus; NAD, nicotinamide adenine dinucleotide; ND, not done; NDI, nuclear division index; NR, not reported; NS, not specified; PFA, paraformaldehyde; PCC, premature chromosome condensation; PI, proliferation index; SCC, squamous cell carcinoma; SCE, sister chromatid exchange; (T), toxicity or cytotoxicity; TK, thymidine kinase; XP, xeroderma pigmentosum; AA8, parental CHO cells; CHO cell mutants deficient in nucleotide excision repair (UV4 & UV5), or transcription-coupled repair (UV61) or crosslink repair-deficient (KO40).

Summary on in vitro genotoxicity of formaldehyde

In vitro genotoxicity of formaldehyde has been reported in several mammalian cell culture systems (see Table A-21). Formaldehyde is mutagenic in several mouse lymphoma cells, Chinese hamster ovary (CHO) and hamster lung epithelial (V79) cells, human lung epithelial carcinoma (A549) cell line, fibroblasts, gastric mucosa cells, and human peripheral blood lymphocytes (PBLs) and lymphoblasts. As shown in Table A-21, several genotoxicity endpoints, such as DNA-protein crosslinks, hydroxymethyl-DNA adducts, single strand breaks, cytogenetic markers, such as micronucleus, chromosomal aberrations, and sister chromatid exchanges, and other genotoxic end points, such as unscheduled DNA synthesis, DNA repair inhibition, and cell transformation have been demonstrated in animal and human cell systems.

Cell lines derived from formaldehyde-induced rat nasal squamous cell carcinomas showed *p53* mutations and the mutant cells were tumorigenic when injected in nude mice, suggesting the mutagenicity and carcinogenicity of formaldehyde. Further, formaldehyde induced deletions and point mutations at the thymidine kinase (*tk*) locus in cultured mouse lymphoma cells and human lymphoblasts or at the hypoxanthine phosphoribosyl transferase (hprt) locus in CHO and V79 cells, and the mutations showed a dose-dependent increase. Further, these mutations contained base substitutions at the AT base pairs at both these loci.

Evidence of formaldehyde-induced genotoxicity was observed in rodent and human cells wherein a dose-dependent increase in DPX formation was reported over a range of formaldehyde concentrations (0.01–0.0625 mM) (see Table A-21). DPX are formed within an hour of exposure and removed within 24 hrs after formaldehyde removal in cultured human cells. The average half-life ($t_{1/2}$) of DPX is 2–3 hours in xeroderma pigmentosum (XP) fibroblasts, 12.5 hours in Ad293

kidney cells and A549 cells, and 18.1 hours (range 1–60 hours) in PBLs. The higher removal time in PBLs is either due to low levels of glutathione in lymphocytes or inefficient repair. Thus, the existing data suggest that repair of DPX depends on the cell type. The removal of DPX is carried out either by spontaneous hydrolysis or other DNA repair processes; however, no difference in DPX removal has been observed between normal human fibroblasts and fibroblasts from XP or Fanconi anemia cell line, suggesting a lack of involvement of nucleotide excision repair in the repair process. In proliferating cells, unrepaired DPX can arrest DNA replication and lead to the induction of other genotoxic effects such as SCEs. Further evidence of DNA reactivity was observed in CHO cells, HeLa cells, and human nasal epithelial cells wherein formaldehyde induced hm-DNA adducts.

Among the other types of genotoxicity, formaldehyde induced SSBs in several mammalian cell systems, including mouse leukemia cells; rat primary hepatocytes, tracheal epithelial cells, and lymphosarcoma cells; and human lung/bronchial epithelial cells, A549 and HeLa cells, skin fibroblasts, and PBLs, within an hour of exposure (see Table A-21). It has been shown that SSBs can be formed directly in lung/bronchial epithelial cells with formaldehyde exposure, independent of DNA repair.

Several studies have demonstrated formaldehyde-induced cytogenetic markers (CAs, MN and SCEs) in different rodent and human primary cells and cell lines (see Table A-21). For example, CAs are induced in CHO cells (normal and DNA repair deficient), V79 cells, and hamster embryo cells, with a dose-dependent increase in human fibroblasts and lymphocytes. Further evidence exists for formaldehyde-induced clastogenic effect as observed by MN induction in V79 cells and a dose-dependent increase in MN induction in both human whole blood cultures and normal and repair deficient fibroblast cells. Furthermore, formaldehyde induced SCEs in CHO cells (normal and repair-deficient) and V79 cells at various concentrations (0.01–0.5 mM). The dose-dependent increase in SCE was higher in mutant CHO cells compared to the normal counterparts, suggesting the importance of DNA repair in SCE removal. Exposure of A549 cells for 1 hour with formaldehyde or co-culturing the exposed A549 cells with unexposed V79 cells beyond 1 hour induces SCE in both cell types, suggesting that formaldehyde is active in the medium for a longer time and continues to induce genotoxicity in spite of the high reactivity of formaldehyde with macromolecules.

In addition, formaldehyde induces DNA repair inhibition in normal as well repair-deficient fibroblasts derived from XP and Fanconi anemia patients. In mouse embryo fibroblasts, formaldehyde acts as a potential initiator with a dose-dependent increase in cell transformation but acts as a weak promoter in hamster kidney cells. Overall, there is significant evidence that formaldehyde is genotoxic and mutagenic in several human and rodent cell culture systems.

A.4.5. Genotoxicity of Formaldehyde in Experimental Animals

In experimental animals, formaldehyde has been shown to induce DNA adducts, DPXs, DDXs, SSBs, cytogenetic alterations, such as, MN, SCEs, CAs, and mutations, as summarized in Table A-22.

DNA reactivity and DNA damage

Formaldehyde is highly DNA reactive. Based on numerous experimental animal studies across several species, exposure has been shown to cause damage at the site of contact and/or portal of entry (POE), including the formation of DNA adducts, DPXs, DDXs, SSBs and other cytogenetic effects (see Table A-22). In addition, some animal studies have reported evidence of effects on DNA at sites distal to the POE; however, these observations were not highly consistent across the available studies (acknowledging that the primary focus of most studies was the POE), and interpretations are complicated by the frequent use of test articles presumed to introduce methanol co-exposure (see Table A-22). This limitation is of significant concern for changes observed outside of the POE.

DNA adducts

Beland et al. (1984) demonstrated the formation of hmDNA mono adducts (e.g., N^6-hmdA) from the in vitro reaction of formaldehyde with calf thymus DNA (see Section A.4.4). The hmDNA adducts are labile in nature and hence they were detected as methylDNA (me-DNA) adducts after chemically reducing them with $NaBH_3CN$ followed by LC/MS analysis (Lu et al., 2011; Moeller et al., 2011; Lu et al., 2010a; Wang et al., 2009a; Wang et al., 2007b). Using [$^{13}CD_2$]-formaldehyde inhalation exposures or orally administered [$^{13}CD_4$]-methanol, one research group has reported the development of an LC/MS method that distinguishes formaldehyde-induced hmDNA mono adducts and DNA-DNA crosslinks originating from endogenous and exogenous exposures in different tissues of rats (Lu et al., 2012b; Lu et al., 2011; Lu et al., 2010a) and monkeys (Moeller et al., 2011). Lu et al. (2010a) exposed F344 rats to a single dose of 12.3 mg/m^3 $^{13}CD_2$-formaldehyde by inhalation for 1 and 5 days. The authors detected three forms of endogenous DNA damage, i.e., the N^2-hmdG and N^6-hmdA mono adducts and dG-CH_2-dG crosslinks, in all tested tissues (nose, lung, liver, spleen, bone marrow, thymus, and blood). The exogenous N^2-hmdG adduct and dG-CH_2-dG crosslinks were detectable only in nasal tissue and their levels increased from 1 day to 5 days of exposure. However, the exogenous N^6-hmdAdo adducts were not detectable in any of the tissues analyzed (Lu et al., 2010a).

The same group of investigators also exposed F344 rats to inhaled [$^{13}CD_2$]-formaldehyde (0.9 to 18.7 mg/m^3) for 6 hours and measured N^2-hmdG adducts in the nasal epithelium (Lu et al., 2011). While both the endogenous and exogenous hmDNA adducts were analyzed in exposed rats, this study did not report the use of unexposed controls. Compared to the ^{13}C-labeled exogenous mono adducts formed by exposures up to 11.2 mg/m^3, endogenous N^2-hmdG adducts formed at levels between 1.7 and over 90-fold higher, showing considerable variation in adduct levels across doses. Although the exogenous N^2-hmdG adducts exhibited a nonlinear increase over the range of concentrations tested, their levels appeared to be above endogenous levels only at the highest formaldehyde concentration tested.

Further, the same group of investigators studied the distribution of hmDNA adducts in Cynomolgus monkeys that were exposed by inhalation to 2.34 or 7.5 mg/m^3 of $^{13}CD_2$-formaldehyde (6 hours/day for 2 days) (Moeller et al., 2011). Endogenous N^2-hmdG mono adducts were detected in the nasal maxilloturbinates and bone marrow, but exogenous DNA adducts were only detectable in the maxilloturbinates. The endogenous tissue levels of hmDNA adducts were 5−10 fold higher than corresponding exogenous adduct levels.

Recently, another study from the same research group examined endogenous and exogenous hm-DNA adducts in rats exposed to low levels of [$^{13}CD_2$]-formaldehyde (1, 30, and 300 ppb) by nose-only inhalation for 28 days (Leng et al., 2019). The authors reported detectable levels of endogenous, but not exogenous hm-DNA adducts in several tissues including those in lower or upper respiratory tract (nasal epithelium, trachea and lung), blood and bone marrow, and in tissues other than respiratory tract, bone marrow and blood cells. Thus, any exogenous formaldehyde-induced hm-DNA adducts are below the limit of detection for exposure concentrations up to 300 ppb (Leng et al., 2019).

In addition to inhalation exposures, hmDNA adducts have been measured after exposure to chemicals (i.e., nitrosamines, methanol) that are metabolized to formaldehyde (Lu et al., 2012b; Wang et al., 2007b). Wang et al. (2007b) have detected the N^6-hmdA adduct in the liver and lung of rats injected subcutaneously with the tobacco-specific nitrosamines, N-nitrosodimethylamine (NDMA), or 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) at 0, 0.025, and 0.01 mmol/kg b.w. doses. The N^6-hmdA adduct showed a dose-response formation with both nitrosamines and was also detected endogenously in saline controls, albeit at low levels. Compared to saline controls, N^6-hmdA levels in exposed rats were 4.5- to 15-fold higher in the liver, and 2.2- to 3.8-fold higher in the lung. Following gavage exposure with 500 and 2,000 mg/kg [$^{13}CD_4$]-labeled methanol, hmDNA adducts were detectable in several tissues of Sprague-Dawley rats, including bone marrow (Lu et al., 2012b). In this study, the authors also analyzed an unexposed control group. A dose-dependent increase in exogenous N^2-hmdG adducts was reported in several tissues including bone marrow, suggesting that exogenous methanol is transported to bone marrow where it is converted to formaldehyde and results in the formation of exogenous hmDNA adducts that are identical to endogenous formaldehyde mono adducts. Interestingly however, the levels of endogenous N^2-hmdG adducts, but not N^6-hmdA adducts, in methanol-exposed animals were significantly increased in several tissues compared to endogenous N^2-hmdG adduct levels in the corresponding tissues of unexposed controls. This observation suggests that exposure to exogenous methanol affects the formation and/or persistence of the endogenous N^2-hmdG, but not N^6-hmdA adducts, which may have also occurred in an earlier rat study that did not report the use of unexposed controls (Lu et al., 2011). From these studies, it appears that hmDNA adducts are likely to be formed in distal tissues when formaldehyde is produced as a metabolite of chemicals such as methanol (Lu et al., 2012b) or from NNK and NDMA (Wang et al., 2007b). Thus, oral exposure to methanol, but not

inhaled formaldehyde, seems to produce formaldehyde-specific adducts in distal tissues of experimental animals.

DNA-protein crosslinks

Several in vivo studies involving rodents and monkeys have demonstrated DPX formation following inhalation exposure to formaldehyde (see Table A-22). In rats, several short- and long-term inhalation exposures of formaldehyde have been shown to induce DPX formation in nasal passages. For example, inhalation exposure to formaldehyde induced DPX in nasal mucosa with a single 3-hour (Casanova and Heck, 1987; Heck and Casanova, 1987) or 6-hour exposure (Casanova et al., 1989; Lam et al., 1985) or 6 hours daily exposure for 2 days (Casanova-Schmitz et al., 1984b; Casanova-Schmitz and Heck, 1983).

DPX levels have been measured from the nasal lateral meatus, medial meatus, and posterior meatus (Casanova et al., 1994) or the entire nasal cavity showing a nonlinear dose-response effect at and above 0.37 mg/m^3 dose (Casanova et al., 1989) after inhalation of ^{14}C-formaldehyde. These sites have been shown to be associated with a high tumor incidence (Morgan et al., 1986b) or cellular proliferation (Monticello et al., 1991; Monticello et al., 1989) in chronic formaldehyde exposure studies in rats.

Casanova-Schmitz and Heck (1983) have reported a significant increase in DPXs in respiratory, but not olfactory mucosa, at ≥7.37 mg/m^3 of formaldehyde exposure of rats with a linear increase in the exposure range of 2.46–36.8 mg/m^3. The inability of this study to detect DPXs at lower levels of formaldehyde exposure is likely due to the protective mechanism of GSH, which catalyzes the oxidative metabolism of formaldehyde to formate. Lam et al. (1985) have shown that co-exposure of rats with 4.6 mg/m^3 acrolein and 7.4 mg/m^3 formaldehyde for 6 hours resulted in higher DPX in the nasal mucosa of rats compared to the rats given formaldehyde alone, suggesting that GSH depletion by acrolein enhanced the macromolecule binding of formaldehyde. The same group in a different study did not detect DPX formation in the olfactory mucosa and bone marrow even at high exposure concentration of 18.42 mg/m^3 (Casanova-Schmitz et al., 1984b).

Casanova and Heck (1987) reported that GSH depletion caused an increase in DPX formation in the IF-DNA of the nasal mucosa of F344 rats when a dual-isotope (^{3}H/^{14}C) method was used. The dual isotope method distinguished between metabolic incorporation and covalent binding of formaldehyde. Formaldehyde is oxidized to formate, losing one hydrogen atom (indicated by a decrease in the ^{3}H/^{14}C ratio), and becomes metabolically incorporated into macromolecules. However, when GSH is not available (depleted), it leaves residual (unoxidized) formaldehyde to covalently bind to DNA, forming DPX. However, the residual formaldehyde may form adducts by reacting with deoxyribonucleosides in the DNA hydrolysates, which could also lead to an overestimation of the amount of DNA-bound formaldehyde. Casanova et al. (1989) used an improved method which is based on the determination of the total ^{14}C-formaldehyde bound to DNA. This study showed that formaldehyde was exclusively bound to IF DNA, indicating the formation of DPXs. Hydrolysis of DPXs in different samples quantitatively released formaldehyde. DPX

formation was detectable at all concentrations (0.37–12.3 mg/m^3 for 6 hours) of formaldehyde exposure. Overall, these studies show that formaldehyde induces DPXs in nasal epithelial cells of rodents. However, there are no published rodent studies that assess DPXs beyond the nasal passages of the upper respiratory tract. Neuss et al. (2010b) did not detect a significant increase in DPX formation, as determined by Comet assay in the bronchoalveolar lavage (BAL) cells of F344 rats exposed up to 18.45 mg/m^3 formaldehyde by whole-body inhalation compared to controls.

DPXs were also found in the nasal mucosa and extranasal tissues of rhesus monkeys exposed to 0.86, 2.45, or 7.36 mg/m^3 formaldehyde 6 hours/day for 3 days (Casanova et al., 1991). These data were used as a basis for cross-species prediction of formaldehyde-induced DPXs in humans. The presence of DPXs in rhesus monkeys confirms formaldehyde's DNA reactivity as a general effect. Additionally, DPXs were detected in the larynx/trachea/carina (pooled sample) and in intrapulmonary airways of monkeys exposed to 2.5 or 7.4 mg/m^3 formaldehyde. These data demonstrate direct effects of formaldehyde on DNA of tissues that correspond to observed tumor sites (e.g., nasal and nasopharynx) in humans.

Recent studies by Lai et al. (2016) have shown that DPXs formed by endogenous formaldehyde were detectable in tissues at the portal of entry (nose) as well as at distal tissues (e.g., blood cells, and bone marrow) in rats or monkeys. However, when either species was exposed to [$^{13}CD_2$]-labeled formaldehyde, exogenous DPXs were detectable only in the respiratory tissues. In rats, exogenous DPXs accumulated over a 28-day period of exposure and remained up to one week after removal of exposure, suggesting that DPXs might be repaired slowly (see Table A-22).

Recently, another study from the same research group examined endogenous and exogenous DPX adducts in rats exposed to low levels of [$^{13}CD_2$]-formaldehyde (1, 30, and 300 ppb) by nose-only inhalation for 28 days (Leng et al., 2019). The authors reported detectable levels of endogenous, but not exogenous DPXs in several tissues including those in lower or upper respiratory tract (nasal epithelium, trachea and lung), blood and bone marrow, and in tissues other than respiratory tract, bone marrow and blood cells. Thus, any exogenous formaldehyde-induced DPX adducts are below the limit of detection for exposure concentrations up to 300 ppb (Leng et al., 2019).

DNA-DNA crosslinks

There is limited evidence showing the formation of DNA-DNA crosslinks (DDX) induced by inhalation exposure to formaldehyde. Lu et al. (2010a) reported dG-CH2-dG crosslinks in the nasal epithelium of F344 rats exposed to 12.3 mg/m^3 formaldehyde for 1 or 5 days (6 hours/day). However, roughly 65% of the dG-CH_2-dG crosslinks were considered artifacts formed during sample workup and storage. Wang et al. (2007b) reported very low levels of dA-CH2-dA crosslinks of formaldehyde in rats exposed to NDMA and NNK, but cautioned that these crosslinks may be generated artifactually upon DNA storage. Thus, the DDX may not be a useful biomarker of formaldehyde exposure.

DNA SSBs by alkaline elution

Formaldehyde has been shown to induce DNA SSBs in few studies involving mice (Wang and Liu, 2006) and rats (Sul et al., 2007; Im et al., 2006), as summarized in Table A-22.

Im et al. (2006) reported a dose-dependent increase in DNA damage as analyzed by the comet assay in both PBLs and livers of Sprague-Dawley rats exposed by inhalation to 6.14 and 12.3 mg/m^3 formaldehyde. In the same strain of rats, Sul et al. (2007) also observed a dose-dependent increase in SSBs in lung epithelial cells following inhalation exposure to 0, 6.15, and 12.3 mg/m^3 formaldehyde for 2 weeks (6 hours/day, 5 days/wk). In a developmental toxicity study, pregnant mice injected i.p. with formaldehyde from gestational days 6 to 19 exhibited DNA damage in maternal as well as fetal liver at 0.2 and 1 mg/kg, respectively (Wang and Liu, 2006).

Cytogenetic markers of genotoxicity

Micronucleus

Few studies examined the effect of formaldehyde exposure on MN induction in rodents by exposing the animals by inhalation, i.p. injection, or gavage as summarized in Table A-22. Inhalation exposure studies in rats were negative, while studies that used formalin by gavage in mice (Ward et al., 1983) and rats (Migliore et al., 1989) were positive for MN formation. Speit and coworkers did not observe MN formation in the peripheral blood cells (Speit et al., 2009) and BAL cells (Neuss et al., 2010b) of F344 rats exposed to 0, 62, 1.23, 7.38, 12.3, and 18.45 mg/m^3 formaldehyde. However, the Neuss et al. (2010b) study did not report the use of a positive control for MN induction, while in the other two studies, the use of cyclophosphamide as a positive control did not appear to induce a high MN count or showed results within the range of control values (Speit et al., 2011b; Speit et al., 2009). Ward et al. (1983) observed aneuploidy and structural chromosomal aberrations (e.g., breaks, exchanges, aberrant chromosomes with and without gaps) in femoral bone marrow cells of mice dosed with formalin (100 mg/kg) or methanol (1,000 mg/kg). The cytogenetic effects seen in bone marrow suggest that the formalin or methanol given by gavage was able to reach bone marrow and induce genotoxicity. Similarly, Migliore et al. (1989) observed MN formation in the gastric epithelial cells of Sprague-Dawley rats exposed to a single dose of formalin (200 mg/kg). Lastly, Liu et al. (2017) have shown that inhalation exposure to formaldehyde in ICR mice for 20 weeks caused a significant increase in the ratio of polychromatic erythrocytes/normochromatic erythrocytes, but not micronuclei induction in bone marrow (Liu et al., 2017).

Sister chromatid exchanges

Few studies examined the effect of formaldehyde exposure on SCEs in mice and rats. Two of the three studies in rats were negative for SCEs in blood cells (Speit et al., 2009; Kligerman et al., 1984), both of these studies used inhalation exposure to 18.45 mg/m^3 formaldehyde for 6 hours/day, 5 days/week for 4 weeks.

In an inhalation study, Brusick (1983) exposed CD-1 mice to target concentrations of 0, 7.38, 14.76, or 30.75 mg/m^3 formaldehyde vapors for 6 hours/day for 4–5 days. Significantly high levels of SCEs/cell were reported in the bone marrow of female mice both at the mid and high concentrations, while the low-concentration group had levels that were not statistically significant from the control group. Thus, formaldehyde exposure has provided equivocal results on the SCEs in rodents.

Chromosomal aberrations

Few studies reported the effect of formaldehyde inhalation on CA induction in rodents and these results were mixed (see Table A-22).

Kligerman et al. (1984) found no difference in the incidence of SCEs or CAs and mitotic index in the PBLs of male and female F344 rats exposed to formaldehyde for 5 days up to 18.45 mg/m^3 dose. Also, Dallas et al. (1992) reported no clastogenic effects in bone marrow of Sprague-Dawley rats exposed at the same concentration of formaldehyde for 8 weeks. However, the authors observed a modest, but statistically significant increase (1.7- to 1.8-fold) in CAs in pulmonary lavage cells at the high dose (18.45 mg/m^3) compared to controls, but not at lower doses [0.61 and 3.7 mg/m^3 (Dallas et al., 1992)].

Speit et al. (2009) investigated the genotoxicity of formaldehyde in peripheral blood samples of Fischer-344 rats exposed to 0 to 18.45 mg/m^3 formaldehyde for 4 weeks (6 hours/day, 5 days/week). Compared to controls, the authors found no significant increase in genotoxicity assays such as the comet assay (with or without γ-irradiation of blood samples), the SCEs assay, and micronucleus test. Earlier studies by Casanova-Schmitz et al. (1984b) showed that formaldehyde does not cause toxicity to bone marrow. Following formaldehyde exposure by i.p. injection in mice, data were negative for CAs in spermatocytes (Fontignie-Houbrechts et al., 1982; Fontignie-Houbrechts, 1981) and polychromatic erythrocytes (Natarajan et al., 1983), while Gomaa et al. (2012) demonstrated an increase in chromosomal aberrations in bone marrow cells of adult male albino rats exposed to formaldehyde at 0.2 mg/kg/day i.p injection for 4 weeks. injection in mice, data were negative for CAs in spermatocytes (Fontignie-Houbrechts et al., 1982; Fontignie-Houbrechts, 1981) and polychromatic erythrocytes (Natarajan et al., 1983), while Gomaa et al. (2012) demonstrated an increase in chromosomal aberrations in bone marrow cells of adult male albino rats exposed to formaldehyde at 0.2 mg/kg/day i.p injection for 4 weeks. Oral administration of formaldehyde to rats showed positive results for CAs in the gastric epithelial cells (Migliore et al., 1989).

Since many leukemogens initiate leukemogenesis by directly damaging the hematopoietic stem cells/hematopoietic progenitor cells (HSP/HPC), Zhao et al. (2020) examined the effect of formaldehyde exposure either in vivo or ex vivo. They exposed either BALB/c mice to 3 mg/m^3 formaldehyde by inhalation for 2 weeks or by ex vivo to cells from bone marrow, lung, nose, and spleen with 0, 50, 100, and 400 μM formaldehyde for 1 hour. Using a myeloid progenitor colony

formation (MPCF) assay, they have shown that formaldehyde exposure caused a decrease in bust-forming unit-erythroid (BFU-E) and colony-forming unit-granulocyte, macrophage (CFU-GM) colonies in all the four tissues from both in vivo and ex vivo (up to 400 μM) exposure to formaldehyde. The authors conclude that their study confirms the presence of HSP/HPC in mouse lung and nose and hypothesize that following formaldehyde-induced DNA damage at the point of entry these damaged stem cells possibly migrate to bone marrow and induce leukemia (Zhao et al., 2020). However, the formaldehyde used in this study was generated from 10% formalin which contains methanol added as a stablizer; it is likely that methanol could also contribute to the outcome, preventing attribution of the results to formaldehyde alone.

Overall, inhalation exposure to formaldehyde has produced mixed and equivocal results in rodents for cytogenetic markers of genotoxicity. Formaldehyde did not induce MN in bone marrow cells of male Sprague-Dawley rats (Dallas et al., 1992) and caused no increase in the frequency of SCEs or CAs and mitotic index in blood lymphocytes of F344 rats of either sex (Kligerman et al., 1984). However, a modest, but statistically significant, increase (1.7- to 1.8-fold) in CAs has been observed in pulmonary lavage cells of Sprague-Dawley rats after exposure to 18.45 mg/m^3 (Dallas et al., 1992) and a significant increase in CAs in bone marrow cells of female Wistar rats exposed to 1.5 mg/m^3 formaldehyde (Kitaeva et al., 1990); however, the latter finding involved methanol co-exposure, reducing confidence in these results. Also, formaldehyde exposure by inhalation in CD-1 mice induced SCEs in bone marrow cells at ≈15 mg/m^3 (Brusick, 1983). Thus, some studies show that inhaled formaldehyde may be able to induce cytogenetic effects in distal tissues with repeated exposures, possibly only at very high formaldehyde concentrations.

Mutations

Formaldehyde exposure has been shown to induce mixed results for mutations in several test systems as summarized in Table A-22. The dominant lethal mutation test has been performed using mice and rats, where males were exposed to formaldehyde or formalin vapors by inhalation or i.p. injection, mated with females, and where mutations were then scored in the offspring. In two of these studies, formaldehyde injected i.p. to CD-1 mice was negative for dominant lethal mutations (Epstein et al., 1972; Epstein and Shafner, 1968), while another study which used a higher dose (50 mg/kg) of formaldehyde showed weakly positive results (Fontignie-Houbrechts, 1981). Specific pathogen-free ICR mice exposed to inhaled formaldehyde were positive for dominant lethal mutations (Liu et al., 2009b). In this study, mutation rates were dose dependent and mainly inherited from the paternal germ line.

Recio et al. (1992) demonstrated point mutations in the GC base pairs of the p53 tumor suppressor gene in 45% (5 out of 11) of the primary nasal squamous cell carcinomas (SCCs) from F344 rats that were chronically (2 years) exposed to 18.45 mg/m^3 formaldehyde. Samples from this study were further analyzed by Wolf et al. (1995) who demonstrated the presence of p53 tumor suppressor protein which correlated with proliferating cell nuclear antigen (PCNA) but not TGF-alpha in the nasal SCCs. However, Meng et al. (2010) failed to detect the p53 mutations in the

nasal mucosa of rats exposed to 0.86 to 18.42 mg/m^3 formaldehyde for 13 weeks. It is likely that the duration of exposure is important for the mutations to occur in these studies. In summary, formaldehyde produced mixed results in the DLM test. Short-term (13-week) exposure of rats to formaldehyde did not produce detectable mutations in the p53 tumor suppressor gene or Ha-ras oncogene; however, a chronic 2-year study resulted in SCC formation and mutations in the GC base pairs of the p53 gene in rats.

Table A-22. Summary of *in vivo* genotoxicity studies of formaldehyde inhalation exposure in experimental animals

Test system	Concentration[a]	Results[b]	Comments	Reference
Mutation				
Evaluations specific to genotoxicity in the upper or lower respiratory tract				
Rats/F344, nasal SCCs	18.45 mg/m^3; HCHO from PFA[c]	+	Inhalation, 6 hrs/d, 5 d/wk, 2 yrs	(Recio et al., 1992)
Rats/F344, nasal SCCs	18.45 mg/m^3; HCHO from PFA	+	Inhalation, 6 hrs/d, 5 d/wk, 2 yrs	(Wolf et al., 1995)
Rats/F344, nasal mucosa	18.45 mg/m^3; HCHO from PFA	–	Inhalation, 6 hrs/d, 5 d/wk,13 wks; Cell proliferation showed a conc.-dependent ↑; significant at 12.3 and 18.45 mg/m^3 exposures	(Meng et al., 2010)
Evaluations specific to genotoxicity to systems other than the respiratory tract, bone marrow, or blood cells				
Rats/Strain not specified - dominant lethal test	1.47 mg/m^3; HCHO (not specified)	(+)	Inhalation, 4 hrs/da for 4 wks	(Kitaeva et al., 1990)
Mice/ICR, specific pathogen-free dominant lethal test	200 mg/m^3; Formalin (37% HCHO w/w aq.sol.)	+	Whole-body inhalation exposure of ♂ mice for 2 hrs; 6 wks postexposure ♂ mated to ♀ at 1:1;	(Liu et al., 2009b)
DNA-protein crosslinks				
Evaluations specific to genotoxicity in the upper or lower respiratory tract				
Monkey/Rhesus nasal turbinates	0.86 mg/m^3; HCHO from PFA	+	Inhalation, 6 hrs; the LEC ↑with the ↑ in distance from the portal of entry; DPX levels show conc.-dependent ↑from 0.86–7.4 mg/m^3, in the order of middle turbinates > lateral wall/septum, nasopharynx > larynx/trachea/carina.	(Casanova et al., 1991)
Monkey/Rhesus nasal, larynx, trachea, & carina	2.5 mg/m^3; HCHO from PFA	+		(Casanova et al., 1991)

Test system	Concentration[a]	Results[b]	Comments	Reference
Monkey/Rhesus maxillary sinuses, lungs	7.4 mg/m^3; HCHO from PFA	+		(Casanova et al., 1991)
Monkeys/Cynomolgus nose	7.4 mg/m^3; HCHO from PFA	+	Inhalation, 6 hrs/d, for 2 d	(Lai et al., 2016)
Rats/F344 nasal mucosa	0.37 mg/m^3; HCHO from PFA	+	Inhalation, 6 hrs; nonlinear conc.-dependent ↑ in DPX between 0.37 to 12.1 mg/m^3	(Casanova et al., 1989)
Rats/F344 nasal mucosa	0.86 mg/m^3; HCHO from PFA	+	Inhalation 6 hrs/d, 5 d/wk, 11 wk + 4 d + 3 hrs (preexposed); or 3 hrs only (naïve); ↑cell proliferation ≥ 7.48 mg/m^3	(Casanova et al., 1994)
Rats/F344 nasal mucosa	2.5 mg/m^3; HCHO from PFA	+	Inhalation, 6 hrs/d, for 2 d; cytotoxicity ≥ 12.3 mg/m^3	(Casanova-Schmitz et al., 1984a)
Rats/F344 nasal mucosa	2.5 mg/m^3; HCHO from PFA	+	Inhalation, 3 hrs/d, for 2 d	(Casanova and Heck, 1987)
Rats/F344 nasal mucosa	2.5 mg/m^3; HCHO from PFA	+	Inhalation, 6 hrs/d; for 7 or 28 d	(Lai et al., 2016)
Rats/F344 nasal mucosa	7.4 mg/m^3; HCHO from PFA	+	Inhalation, 6 hrs/day, for 2 days	(Casanova-Schmitz and Heck, 1983)[b]
Rats/F344 nasal mucosa	7.4 mg/m^3; HCHO from PFA	+	Inhalation, 6 hrs; co-exposure to 2 ppm acrolein caused a significant ↑ in toxicity and DPX formation	(Lam et al., 1985)
Rats/F344 nasal mucosa	18.45 mg/m^3; HCHO from PFA	+	Inhalation, 6 hrs/d; for 1, 2, and 4 d	(Lai et al., 2016)
Rats/F344 olfactory mucosa	18.45 mg/m^3; HCHO from PFA	–	Inhalation, 6 hrs/d, for 2 d	(Casanova-Schmitz et al., 1984a)
	36.9 mg/m^3; HCHO from PFA	–	Inhalation, 6 hrs/d, for 2 d	(Casanova-Schmitz and Heck, 1983)[b]
Rats/F344, nasal epithelium, trachea, lung	0.0012, 0.0369, 0.369 mg/m^3 [$^{13}CD_2$]-HCHO	-	Inhalation, nose-only, 6 h/d, 28 d	(Leng et al., 2019)
Rats/F344 BAL cells	18.45 mg/m^3; HCHO from formalin vapors	–	Inhalation, 6 hrs/d, 5 d/wk, for 4 wks	(Neuss et al., 2010)

Test system	Concentration[a]	Results[b]	Comments	Reference
Mice/BalbC lung	3.0 mg/m^3; HCHO vapor from 10% formalin	–	Inhalation, nose-only; 8 hrs/d for 7 d;	(Ye et al., 2013)
Evaluations specific to genotoxicity in cells of the blood and bone marrow				
Monkeys/Cynomolgus bone marrow, PBMC	7.4 mg/m^3; HCHO from PFA	-	Inhalation, 6 hrs/d, for 2 d	(Lai et al., 2016)
Rats/F344 bone marrow	12.43 mg/m^3; HCHO from PFA	–	Inhalation, 3 hrs/d, for 2 d	(Casanova and Heck, 1987)
Rats/F344 bone marrow	18.45 mg/m^3; HCHO from PFA	–	Inhalation, 6 hrs/d, for 2 d	(Casanova-Schmitz et al., 1984a)
Rats/F344 bone marrow, PBMC	18.45 mg/m^3; HCHO from PFA	–	Inhalation, 6 hrs/d; for 1, 2, and 4 d	(Lai et al., 2016)
Rats/F344, bone marrow, PB MC	0.0012, 0.0369, 0.369 mg/m^3 [$^{13}CD_2$]-HCHO	-	Inhalation, nose-only, 6 h/d, 28 d	(Leng et al., 2019)
Rats/F344 peripheral blood	18.45 mg/m^3; HCHO from formalin vapors	–	Inhalation, 6 hrs/d, 5 d/wk, for 4 wks	(Speit et al., 2009)
Mice/BalbC bone marrow	1.0 mg/m^3; HCHO vapor from 10% formalin	+	Inhalation, nose-only; 8 hrs/d for 7 d; dose-dependent ↑ in DPX	(Ye et al., 2013)
Mice/BalbC PBM cells	3.0 mg/m^3; HCHO vapor from 10% formalin	+	Inhalation, nose-only; 8 hrs/d for 7 d; dose-dependent ↑ in DPX	(Ye et al., 2013)
Evaluations specific to genotoxicity in systems other than the respiratory tract, bone marrow or cells of the blood				
Monkeys/Cynomolgus liver	7.4 smg/m^3; HCHO from PFA	-	Inhalation, 6 hrs/, for 2 d	(Lai et al., 2016)
Rats/F344, olfactory bulbs, liver, hippo campus, cerebellum	0.0012, 0.0369, 0.369 mg/m^3 [$^{13}CD_2$]-HCHO	-	Inhalation, nose-only, 6 h/d, 28 d	(Leng et al., 2019)
Mice/Kunming kidney & testes	0.5 mg/m^3; HCHO vapor from 10% formalin	+	Inhalation, 72 hrs continuous exposure	(Peng et al., 2006)
Mice/Kunming liver	1.0 mg/m^3; HCHO vapor from 10% formalin	+	Inhalation, 72 hrs continuous exposure	(Zhao et al., 2009; Peng et al., 2006)
Mice/BalbC spleen, testes	1.0 mg/m^3; HCHO vapor from 10% formalin	+	Inhalation, nose-only; 8 hrs/d for 7 d; dose-dependent ↑ in DPX	(Ye et al., 2013)
DNA adducts				
Evaluations specific to genotoxicity in the upper or lower respiratory tract				
Monkey/Cynomologus maxilloturninate	2.33 mg/m^3; HCHO (not specified)	+	Inhalation, 6 hrs/d, for 2 d; conc.-dependent ↑ in exogenous adducts	(Moeller et al., 2011)

Test system	Concentration[a]	Results[b]	Comments	Reference
Monkeys/Cynomolgus - nasal dorsal mucosa, nasopharynx, nasal septum, nasal posterior maxillary	7.5 mg/m^3; HCHO from PFA	+	Inhalation, 6 hrs/d, for 2 d	(Yu et al., 2015b)
Monkeys/Cynomolgus - trachea carina, trachea proximal	7.5 mg/m^3; HCHO from PFA	–	Inhalation, 6 hrs/d, for 2 d	(Yu et al., 2015b)
Rats/F344 nasal epithelium	0.86 mg/m^3; HCHO from PFA	+	Inhalation, for 6 hrs; conc.-dependent ↑ in exogenous adducts	(Lu et al., 2011)
Rats/F344 nasal epithelium	2.46 mg/m^3; HCHO from PFA	+	Inhalation, 6 hrs/d, for 7, 14, 21, or 28 d; recovery for 6, 24, 72, or 168 hrs; exposure-dependent ↑ hmdG mono adducts	(Yu et al., 2015b)
Rats/F344 -nasal epithelium	12.3 mg/m^3; 20% HCHO in water	+	Inhalation, 1 and 5 d; exposure-dependent ↑ in exogenous hmdG adduct and dG-dG crosslinks	(Lu et al., 2010a)
Rats/F344 lung	12.3 mg/m^3; HCHO from PFA	–	Inhalation, 1 and 5 d	(Lu et al., 2010a)
Rats/F344, nasal epithelium, trachea, lung	0.0012, 0.0369, 0.369 mg/m^3 [$^{13}CD_2$]-HCHO	-	Inhalation, nose-only, 6 h/d, 28 d	(Leng et al., 2019)
Evaluations specific to genotoxicity in cells of the blood and bone marrow				
Monkey/Cynomologus bone marrow	2.33 mg/m^3; HCHO (not specified)	–	Inhalation, 6 hrs/d, for 2 d	(Moeller et al., 2011)
Monkeys/Cynomolgus bone marrow, white blood cells	7.5 mg/m^3; HCHO from PFA	–	Inhalation, 6 hrs/d, for 2 d	(Yu et al., 2015b)
Rats/F344 white blood cells and bone marrow cells	12.3 mg/m^3; HCHO from PFA	–	Inhalation, 1 and 5 d	(Lu et al., 2010a)
Rats/F344, bone marrow, PB MC	0.0012, 0.0369, 0.369 mg/m^3 [$^{13}CD_2$]-HCHO	-	Inhalation, nose-only, 6 h/d, 28 d	(Leng et al., 2019)
Evaluations specific to genotoxicity in systems other than the respiratory tract, bone marrow or cells of the blood				
Rats/F344 thymus, lymph nodes, trachea, lung, spleen, kidney, liver, brain	2.46 mg/m^3; HCHO from PFA	–	Inhalation, 6 hrs/d, for 28 d	(Yu et al., 2015b)
Rats/F344 liver, spleen, thymus	12.3 mg/m^3; HCHO from PFA	–	Inhalation, 1 and 5 d	(Lu et al., 2010a)
Rats/F344, olfactory bulbs, liver, hippo campus, cerebellum	0.0012, 0.0369, 0.369 mg/m^3 [$^{13}CD_2$]-HCHO	-	Inhalation, nose-only, 6 h/d, 28 d	(Leng et al., 2019)
Chromosomal aberrations				
Evaluations specific to genotoxicity in the upper or lower respiratory tract				

Test system	Concentration[a]	Results[b]	Comments	Reference
Rats/SD Pulmonary lavage cells	18.45 mg/m^3; HCHO from PFA	+	Inhalation, whole body; 6 hrs/d, 1 or 8 wks	(Dallas et al., 1992)
Evaluations specific to genotoxicity in cells of the blood and bone marrow				
Rats/Wistar Bone marrow	0.49 mg/m^3; HCHO (not specified)	+	Inhalation, 4 hrs/d, 4 mos	(Kitaeva et al., 1990)
Rats/SD Bone marrow	18.45 mg/m^3; HCHO from PFA	–	Inhalation, whole body; 6 hrs/d, 1 or 8 wks	(Dallas et al., 1992)
Rats/F344 Peripheral blood cells	18.45 mg/m^3; HCHO from PFA	–	Inhalation, 6 hrs/d, 5 d/wk, for 4 wks	(Speit et al., 2009)
Rats/F344 Lymphocytes	18.45 mg/m^3; HCHO from PFA	–	Inhalation, 6 hrs/d, 5 d; no significant dose-related effect on mitotic activity	(Kligerman et al., 1984)
Mice/CD-1, male & female, Bone marrow cells	30.75 mg/m^3; HCHO from PFA	–	Inhalation, 6 hrs/d, 4–5 d	(Brusick, 1983)
Mice/BALB/c, bone marrow – hematopoietic stem and progenitor cells	3 mg/m^3, HCHO from 10% formalin	+	Inhalation, 8 h/d, 5d/wk, 2 wks	(Zhao et al., 2020)
Micronucleus				
Evaluations specific to genotoxicity in the upper or lower respiratory tract				
Rats/F344 BAL cells	18.45 mg/m^3; HCHO from formalin vapors	–	Inhalation, 6 hrs/d, 5 d/wk, for 4 wks; positive control was not used for the assay	(Neuss et al., 2010a)
Evaluations specific to genotoxicity in cells of the blood and bone marrow				
Rats/Outbred white polychromatophylic erythrocytes (bone marrow)	12.8 mg/m^3, commercial formaldehyde	+	Inhalation; whole-body exposure; 4 hrs/d, 5 d/wk	(Katsnelson et al., 2013)
Rats/F344 -peripheral blood	18.45 mg/m^3; HCHO from formalin vapors	–	Inhalation, 6 hrs/day, 5 days/wk, for 4 wks	(Speit et al., 2009)
Mice/male ICR bone marrow cells	20 mg/m^3 36.5%-38% HCHO in water (formalin)	+	Inhalation, 2 hrs/d for 15 d	(Yu et al., 2014a)
Mice/ICR, bone marrow cells	1, 10 mg/m^3, HCHO source not reported	-	Inhalation, 2 h/d, 20 wks; micronucleus	(Liu et al., 2017)
Single strand breaks				
Evaluations specific to genotoxicity in the upper or lower respiratory tract				
Rats/SD lung epithelial cells	6.14 mg/m^3; HCHO (commercial)	+	Inhalation, 6 hrs/d, 5 d/wk for 2 wks; ↑cytotoxicity (lipid peroxidation & protein carbonyl oxidation) observed at 18.42 mg/m^3	(Sul et al., 2007)
Evaluations specific to genotoxicity in blood cells				

Test system	Concentration[a]	Results[b]	Comments	Reference
Rats/SD, PBLs	6.14 mg/m^3; HCHO (commercial)	+	Inhalation, 5 d/wk for 2 wks	(Im et al., 2006)
Evaluations specific to genotoxicity in systems other than the respiratory tract, bone marrow or blood cells				
Rats/SD, liver	6.14 mg/m^3; HCHO (commercial)	+	Inhalation, 5 d/wk for 2 wks	(Im et al., 2006)
Sister chromatid exchanges				
Evaluations specific to genotoxicity in cells of the blood and bone marrow				
Rats/F344 Lymphocyte	18.45 mg/m^3; HCHO from PFA	−	Inhalation, 6 hrs/d, 5 d; no significant dose-related effect on mitotic activity	(Kligerman et al., 1984)
Rats/F344 Peripheral blood cells	18.45 mg/m^3; Formalin vapors	−	Inhalation, 6 hrs/d 5 d/wk, for 4 wks	(Speit et al., 2009)
Mice/CD-1, male & female Bone marrow cells	14.76 mg/m^3; HCHO from PFA	−, +	Inhalation, 6 hrs/d, 5 d; ♂ mice: −ve; ♀ mice: +ve; conc.-dependent ↑ in SCEs	(Brusick, 1983)

Gray shading indicates experiments examining tissues or cells outside of the upper respiratory tract that are assumed to have included co-exposure to methanol, and are thus may be less reliable.

[a]Lowest effective concentration (LEC) for positive results or highest ineffective concentration tested (HIC) for negative or equivocal results.

[b]+ = positive; − = negative; (+), equivocal.

[c]Thermal depolymerization of paraformaldehyde (PFA) or freshly prepared formalin (no methanol) are the preferred test article methods. Generation of formaldehyde from formalin, uncharacterized aqueous solutions (noted as **not specified**), or an unspecified source (also noted as **not specified**) is assumed to involve co-exposure to methanol, and the evidence is less reliable.

HCHO, formaldehyde; PFA, paraformaldehyde; hmDNA, hydroxymethylDNA; SCE, sister chromatid exchange; SCC, squamous cell carcinoma; hmdA, hydroxymethyl deoxyadenosine; hmdG, hydroxymethyl deoxyguanosine; MN, micronucleus.

Part of the data adapted from NTP (2010).

Table A-23. Summary of in vivo genotoxicity studies of formaldehyde exposure by intraperitoneal and oral routes of exposure in experimental animals

Test system	Concentration[a]	Results[b]	Comments	Reference
Mutation				
Rats/Albino Spermatocyte; DLM	0.125 mg/kg; test article: **37% HCHO (+ 10% methanol)**	+	i.p., ♂ given 5 daily doses and mated to ♀; dose-dependent ↑ in DLM index; effects greater with shorter time gap postexposure	(Odeigah, 1997)
Mice/CD-1 DLM test	20 mg/kg HCHO; test article: **Not Specified**	−	i.p. injection to ♂; mated to ♀ and autopsied 13 d past mid-wk of mating	(Epstein and Shafner, 1968)
DNA-protein crosslinks				
Rats/F344 tracheal implants	0.01% HCHO in PBS; test article: **Not Specified**	+	instillation, twice weekly for 2, 4, or 8 wks	(Cosma et al., 1988)

Test system	Concentration[a]	Results[b]	Comments	Reference
Mice/NS liver (Fetal) [Chinese lang-English Abstract]	0.2 mg/kg; test article: **HCHO (not specified)**	+	i.p. injection to pregnant mice from GD 6 to 19	(Wang and Liu, 2006)
Mice/NS Liver (maternal) [Chinese lang-English Abstract]	20 mg/kg; test article: **HCHO (not specified)**	−	i.p. injection to pregnant mice from GD 6 to 19	(Wang and Liu, 2006)
Chromosomal aberrations				
Mice/CBA femoral polychromatic erythrocytes	25 mg/kg; test article: HCHO (PFA in water)	−	i.p. injections (two) within 24 hr interval; cells sampled 16 and 40 hrs post 2nd inj.	(Natarajan et al., 1983)
Mice/Q strain Spermatocytes	50 mg/kg; test article: **HCHO (35% sol.)**	−	i.p. injection, single	(Fontignie-Houbrechts, 1981)
Mice/Q strain Spermatogonia	30 mg/kg; test article: **HCHO (commercial)**	−	i.p., 35% HCHO solution + 90 mg/kg H_2O_2	(Fontignie-Houbrechts et al., 1982)
Rats/SD gastric epithelial cells (stomach, duodenum, ileum, colon)	200 mg/kg; test article: HCHO (in water)	+	p.o., 16, 24, or 30 hrs; time-dependent ↑ in CA in all tissues; toxic at 30 hrs; no significant change in mitotic index	(Migliore et al., 1989)
Mice/B6C3F1-bone marrow	100 mg/kg; test article: **formalin**; or 1,000 mg/kg methanol	+	Gavage, single exposure; HCHO and methanol showed 21− and 15−fold increase compared to controls, respectively	(Ward et al., 1983)
Rats (male albino), bone marrow cells	0.2 mg/kg/day; test article: HCHO (**source not specified)**	+	i.p injection, single injection for 4 wks	(Gomaa et al., 2012)
Micronucleus				
Mice/CBA femoral polychromatic erythrocyte and spleen cell	25 mg/kg; test article: HCHO (PFA in water)	−	i.p. injections (two) of HCHO solution within 24 hr interval; cells sampled 16 and 40 hrs post 2nd inj.	(Natarajan et al., 1983)
Mice/NMRI bone marrow	30 mg/kg; test article: **HCHO (commercial)**	−	i.p. injection, single	(Gocke et al., 1981)
Mice/CD-1 reticulocytes	30 mg/kg; test article: **HCHO (35%)**	−	i.v. two injections; sampled 24, 48, or 72 hrs after exposure	(Morita et al., 1997)
Mice/CD-1 bone marrow or peripheral blood	200 mg/kg; test article: **35% HCHO**	−	Gavage twice (bone marrow) or once (peripheral blood); all mice killed at 300 mg/kg dose	(Morita et al., 1997)
Rats/SD gastric epithelial cells (stomach, duodenum, ileum, colon)	200 mg/kg; test article: HCHO (in water)	+	p.o., 16, 24, or 30 hrs; time-dependent ↑ in MN in all tissues; toxic at 30 hrs; no significant change in mitotic index	(Migliore et al., 1989)

[a]Lowest effective concentration (LEC) for positive results or highest ineffective concentration (HIC) tested for negative or equivocal results.
[b]+ = positive; − = negative; (+), equivocal.
[c]Thermal depolymerization of paraformaldehyde (PFA) or freshly prepared formalin (no methanol) are the preferred test article methods. Generation of formaldehyde from formalin, uncharacterized aqueous solutions (noted as **not specified**), or an unspecified source (also noted as **not specified**) is assumed to involve co-exposure to methanol, and the evidence is less reliable.
HCHO, formaldehyde; PFA, paraformaldehyde; DLM, dominant lethal mutation; i.p., intra peritoneal; i.v., intra venous; GD, gestation day; MN, micronucleus;
Part of the data adapted from NTP (2010).

Summary of in vivo genotoxicity studies of formaldehyde by routes of exposure in experimental animals

Formaldehyde reacts with cellular macromolecules at the portal of entry causing genotoxicity. Genotoxicity of inhaled formaldehyde involves direct interaction with DNA inducing DNA-protein crosslinks and/or hydroxymethylDNA adducts or DNA mono adducts, single strand breaks, micronuclei, and chromosomal aberrations in nasal passages of experimental animals. DPX are formed predominantly by crosslinking of the epsilon-amino groups of lysine and the exocyclic amino groups of DNA, especially the N-terminus of histone. Due to the differences in the anatomy of nasal passages and breathing patterns of rats and monkeys, the location of DPX formation differs. Over a range of 0.86 to 7.37 mg/m^3, formaldehyde-induced DPX levels showed concentration-dependent increase in monkey respiratory tract in the order of middle turbinates > anterior lateral wall/septum > maxillary sinuses and lungs. Thus, the lowest effective concentration (LEC) being higher with increase in the anatomical distance from the portal of entry. Furthermore, these anatomical sites are known to be associated with formaldehyde-induced proliferative response in monkeys. In rats, DPX formation showed concentration dependence between 0.37−12.1 mg/m^3 formaldehyde, which was nonlinear with a sharp increase above 4.9 mg/m^3. With exposures up to 28 days, DPXs were shown to accumulate and persisted for an additional 7 days at a concentration of 2.5 mg/m^3. In addition, DPX formation was six-fold higher in the lateral meatus compared to the medial and posterior meatus, corresponding, respectively, to high and low tumor incidence sites in rats. DPXs were not detected in olfactory mucosa, bronchoalveolar lavage (BAL) cells of rats or in lungs of mice exposed to formaldehyde. DPXs (from exogenous formaldehyde) also were not detected in bone marrow and peripheral blood monocyte cells (rats and monkeys) and liver (monkeys) following inhalation exposure. Since DPXs are likely to induce replication errors, they have been considered to be a marker of mutagenicity. The repair of DPX in eukaryotes appears to depend on the dose and duration of formaldehyde exposure. The overall evidence indicates that the DPXs are markers of exposure as well as genotoxic endpoints.

HydroxymethylDNA adducts in experimental animals can result from DNA reacting with endogenously-produced or exogenous formaldehyde. Mono adducts formed from endogenous formaldehyde (produced during normal cellular metabolism) are distinguished from those formed by exogenous exposure using stable isotope (^{13}C)-labeled formaldehyde coupled with sensitive MS

techniques. Inhaled formaldehyde induces N2-hmdG adducts in the nasal epithelium of F344 rats, but not in distal tissues, and the adduct levels are associated with concentration and duration of exposure. In rhesus monkeys, formaldehyde induces N2-hmdG adducts in the maxilloturbinates, and the mono adduct levels are associated with the exposure concentration of formaldehyde. Endogenous N2-hmdG mono adducts and dG-dG crosslinks are also detected in rats and monkeys, but in all experimental animals exposed exogenously to formaldehyde by inhalation, N2-hmdG adducts were only elevated in nasal passages, not in tissues beyond the portal of entry. However, formaldehyde-specific hmDNA adducts have been detected in rodent tissues distal to the portal of entry when the animals were exposed to methanol or nitrosamines, which are known to release formaldehyde as a metabolic intermediate in vivo. These studies suggest the lack of transport of formaldehyde beyond the portal of entry when given by inhalation in animals. Although the hmDNA adducts are considred to be genotoxic endpoints of formaldehyde exposure, their mutagenicity has not been enstablished.

There is limited evidence about mutagenicity of formaldehyde in experimental animals. Formaldehyde did not induce mutations in the nasal mucosa of rats with inhalation exposure to 18.5 mg/m^3 for 13 weeks, but there are no available studies involving longer periods of exposure. However, formaldehyde inhalation exposure caused other genotoxic endpoints, including chromosomal aberrations and single strand breaks but not micronuclei in cells of respiratory system.

Twelve out of 17 that analyzed formaldehyde-induced genotoxic endpoints in bone marrow or blood cells were negative. Conflicting results have been obtained in terms of source of formaldehyde. Formaldehyde derived from paraformaldehyde or commercial formalin was negative for DPX formation in bone marrow and peripheral blood cells, although one recent study, which used 10% formalin as a source of formaldehyde, induced DPX in bone marrow and peripheral blood mononuclear cells. Formaldehyde did not induce hmDNA adducts in the bone marrow of monkeys and rats, suggesting that inhaled exogenous formaldehyde may not be transported to the tissues distal to the portal of entry. Formaldehyde failed to induce CAs in 4/5 studies in the bone marrow or peripheral blood cells of rats and mice (see Table A-22), although one study detected CAs in bone marrow of rats. Limited available evidence shows that inhaled formaldehyde did not induce micronuclei in the peripheral blood cells of rats, but was positive for inducing SSBs in peripheral blood and bone marrow cells and produced mixed results on SCE formation. The above studies clearly indicate the complexicity of data analyses with contradicting results in the same assay sytem, type of exposure, and/or methodology utilized.

Formaldehyde produced mixed results in tissues other than the respiratory and hematopoietic systems (see Table A-23). Three studies demonstrated DPX formation in mouse kidney, testes, liver and spleen when 10% formalin was used as a source of formaldehyde. Inhaled formaldehyde did not induce hmDNA adducts in the liver, spleen, and thymus of rats, but SSBs were detectable in the liver of rats following inhalation exposure.

Several studies evaluated the genotoxicity and mutagenicity of formaldehyde by routes other than inhalation exposure and reported mixed results (see Table A-23), suggesting that formaldehyde induced genotoxicity might depend on the route of exposure and formulation of formaldehyde administered.

A.4.6. Genotoxic Endpoints in Humans

A large set of research studies in several countries, involving different exposure settings, found that exposure to formaldehyde is associated with damage or changes to human DNA that inform mechanisms of carcinogenesis. These studies have observed increased levels of DNA damage, DNA-protein crosslinks, and chromosomal breaks in buccal and nasal epithelial cells, and peripheral blood lymphocytes. Chromosomal damage, manifested as an increased frequency of different types of chromosomal aberrations, has been reported. It has been shown that increased frequency of chromosomal aberrations and micronuclei are associated with increased cancer mortality, and these endpoints are considered by EPA to be highly relevant to the assessment of genotoxicity in humans (Bonassi et al., 2011; Bonassi et al., 2008; Bonassi et al., 2007; U.S. EPA, 2005; Bonassi et al., 2004b). Single strand breaks in DNA, indicating genetic instability also are considered by EPA to be highly relevant to the assessment of genotoxicity for humans. However, an increased level of sister chromatid exchange in peripheral lymphocytes has not been found to be associated with cancer mortality in a large collaborative evaluation (Bonassi et al., 2004a). Although sister chromatid exchange is an indication of genotoxicity, this endpoint is considered to be less relevant as a predictor of cancer risk. The studies that reported SCE results were evaluated and are summarized in tables but are not synthesized because of the large amount of evidence for other genotoxicity endpoints.

EPA evaluated the studies, focusing on study design, comparison groups, assessment of exposure and cytogenetic endpoints, and analytic methods. As discussed in this synthesis, although the entire set of studies contributed to the assessment, those with the stronger study designs and methods, and which provided adequate details, were given more weight. Most of the studies reporting on measures of genotoxicity did not describe the details of population selection, recruitment, and participation, which makes it difficult to evaluate potential selection bias. However, most did report the population source(s), and since knowledge of a person's status regarding these endpoints would not be a factor in his or her decision to participate, the reporting deficiency is likely not a serious limitation.

Chromosomal Aberrations in Peripheral Blood Lymphocytes

A total of 16 studies were available that evaluated chromosomal aberrations in peripheral blood lymphocytes (PBLs) or less differentiated subsets among individuals in a variety of exposure settings, including students in anatomy and embalming courses, workers in industrial settings, and workers in pathology laboratories (Table A-24). Average formaldehyde concentrations in these occupational settings generally were above 0.1 mg/m^3, although two studies evaluated

chromosomal aberrations among groups exposed to lower average concentrations (Santovito et al., 2011; Pala et al., 2008). Study results were heterogeneous, and the studies were variable in their study designs and reporting detail. Several did not state whether sample analysis was blinded with respect to exposure status, did not provide demographic information on exposed and referent groups to support assertions of similarity, had extremely small sample sizes (N <15), or incubated cells for longer than 48–50 hours (thus not restricting to M_1 metaphases, and/ or did not describe their approach to data analysis: (Gomaa et al., 2012; Lazutka et al., 1999; He et al., 1998; Kitaeva et al., 1996; Vasudeva and Anand, 1996; Vargová et al., 1992; Thomson et al., 1984; Fleig et al., 1982; Suskov and Sazonova, 1982). Nine publications for 8 occupational groups provided detailed descriptions of study methods and important attributes of the exposed and referent groups (Costa et al., 2015; Lan et al., 2015; Santovito et al., 2014; Musak et al., 2013; Santovito et al., 2011; Jakab et al., 2010; Zhang et al., 2010; Pala et al., 2008; Bauchinger and Schmid, 1985).

Formaldehyde was associated with a higher prevalence of chromosomal aberrations among workers in pathology laboratories (Costa et al., 2015; Musak et al., 2013; Santovito et al., 2011; Jakab et al., 2010); these effects included chromatid-type aberrations (Costa et al., 2015; Jakab et al., 2010), chromosome-type aberrations (Costa et al., 2015; Musak et al., 2013), chromosomal exchange (Musak et al., 2013), and premature centromere division (Jakab et al., 2010). Costa et al. (2015) also reported an increase in aneuploidies and in the number of aberrant and multiaberrant cells. In one study of paper makers, formaldehyde exposure was associated with dicentrics and centric rings (Bauchinger and Schmid, 1985). Average 8-hour TWA formaldehyde concentrations of 0.32, 0.47, and 0.9 mg/m^3 were associated with a 1.7–1.9-fold increase in total chromosomal aberrations among exposed groups (Costa et al., 2015; Musak et al., 2013; Jakab et al., 2010). An increased mean number of chromosomal aberrations per cell was significantly associated with an 8-hour TWA concentration of 0.07 mg/m^3 among pathologists compared to unexposed hospital workers exposed to 0.04 mg/m^3 by Santovito et al. (2011). One well-conducted study did not observe associations (Pala et al., 2008), possibly because the group of laboratory workers was exposed to very low formaldehyde concentrations (75% of workers at < 0.026 mg/m^3). Another study in nurses found no differences with their referent group, although this group likely experienced a wide variation in the intensity of their formaldehyde exposure, and no formaldehyde measurements were conducted (Santovito et al., 2014). An increased frequency of chromosomal aberrations or aberrant cells was also found in a few studies that incubated cell cultures for a longer period (72 hours) (Gomaa et al., 2012; Lazutka et al., 1999; Kitaeva et al., 1996), but not by all (Vasudeva and Anand, 1996; Fleig et al., 1982). Incubation times longer than required to achieve first generation metaphase would be expected to result in greater heterogeneity in the aberration frequencies detected.

Zhang et al. (2010), using fluorescence in situ hybridization techniques, observed an increased level of chromosome aneuploidy (monosomy 7 and trisomy 8) in cultured CFU-GM colony cells in a small group of highly exposed formaldehyde-melamine production workers

(n = 10) compared to a referent group matched by age and gender (n = 12). Although only a small number of workers were evaluated, this report provided complete details on study design, participation, population characteristics, exposure measurements, cytogenetic analyses, and data analysis and results. Subsequently, a larger group of the same cohort (n = 29 exposed, n = 23 referent) were included in a chromosome-wide evaluation of aneuploidy, again using cultured CFU-GM colony cells (Lan et al., 2015). An elevated risk ratio for monosomy, trisomy, and tetrasomy was found in several chromosomes, including chromosomes 5 and 7, a finding that was predicted a priori. In addition, investigators reported an increased frequency of structural chromosome aberrations in chromosome 5 (IRR 4.15, 95% CI 1.20−14.35). Gentry et al. (2013) reported on analyses using data on the cohort studied by Zhang et al. (2010) and noted that few of the DNA analyses scored 150 or more cells per individual as specified by the study protocol. Although the pilot study methods were criticized for not adhering to the assay protocol (Gentry et al., 2013), a clarification of the assay protocol was provided by the investigators with a description of how the study adhered to it (Rothman et al., 2017). The criticism by Gentry et al. (2013) applied to both the exposed and unexposed groups; thus, no bias should have occurred. Analyzing fewer cells per individual may have increased the variability in the prevalence estimates of aneuploidy, which may have attenuated the measures of association. Although the chromosome anomalies may have arisen either in vivo or during the in vitro cell culture period (Gentry et al., 2013), there was a significant increase in the exposed workers compared to the referent group, indicating a formaldehyde-associated tendency toward aneuploidy or other chromosomal abberations. Median formaldehyde concentrations measured in the exposed and referent groups were 1.7 mg/m^3 and 0.032 mg/m^3, respectively. Personal exposure monitoring was conducted for several other chemical exposures, including chloroform, methylene chloride, tetrachloroethylene, trichloroethylene, benzene, or other hydrocarbons, which were not detected. Statistical models were adjusted for potential confounders including age, gender, recent infection, body mass index, and current tobacco, alcohol, and medication use.

The differences in lymphocyte subset levels between exposed and unexposed workers reported by Zhang et al. (2010) were challenged by Mundt et al. (2017) in a reanalysis who did not find evidence of an exposure-response trend within the exposed group, although the difference between unexposed and exposed subjects was reconfirmed. Rothman et al. (2017) also responded to the critique by Mundt et al. (2017) explaining that the exposure levels in the exposed group were relatively homogenous and the study was not designed to provide a range of exposures wide enough to evaluate exposure-response relationships given the expected effect size and sample size in the study. Overall, the evidence from the set of studies in which there is higher confidence are consistent with the finding that formaldehyde exposure is associated with chromosomal aberrations in peripheral blood lymphocytes.

Micronuclei

An increase in micronuclei in buccal mucosa, nasal mucosal cells and peripheral blood lymphocytes (PBLs) was associated with formaldehyde exposure in a large number of studies (see Table A-24). Micronuclei were reported in a diverse set of exposed populations including plywood production workers, formaldehyde production and other chemical workers, pathologists and other laboratory workers, and anatomy and mortuary lab students, and were observed at average concentrations of 0.1 mg/m^3 (Wang et al., 2019; Ballarin et al., 1992), 0.2 mg/m^3 (Costa et al., 2019; Ladeira et al., 2011), and 0.5 mg/m^3 (Costa et al., 2013; Costa et al., 2011; Costa et al., 2008; Ying et al., 1997). Micronuclei in peripheral lymphocytes and exfoliated cells are considered biomarkers of genotoxic events and chromosomal instability, including errors in DNA repair mechanisms, dysfunction or lack of telomeres, and other failures during DNA replication and repair processes (Bonassi et al., 2011). Micronuclei in PBL is a validated predictor of cancer risk in epidemiology studies (Bonassi et al., 2007). Studies of exposure to formaldehyde over a short duration found no changes in micronucleus frequency in nasal mucosal cells (Zeller et al., 2011), buccal mucosal cells (Speit et al., 2007a, 4-hour exposures for 10 days, 4-hour exposures for 10 days) or peripheral blood lymphocytes (Lin et al., 2013, 8-hour cross-shift change, 8-hour cross-shift change).

Measurements in exfoliated buccal cells (EBC) revealed a consistently increased frequency of micronuclei or binucleated cells among exposed individuals (Costa et al., 2019; Aglan and Mansour, 2018; Peteffi et al., 2015; Ladeira et al., 2011; Viegas et al., 2010; Burgaz et al., 2002; Ying et al., 1997; Titenko-Holland et al., 1996; Suruda et al., 1993). Differences were reported using various study designs, including changes in anatomy and embalming students before and after lab courses and prevalence surveys comparing exposed workers and referent groups. Generally, differences were observed at formaldehyde exposure levels averaging 0.2 mg/m^3 and above. Micronuclei frequencies were greater by 1.5 to 6-fold in exposed workers with mean formaldehyde concentrations of 0.2 to 0.5 mg/m^3 compared to referent groups (Costa et al., 2019; Ladeira et al., 2011; Viegas et al., 2010). Most of the studies of micronuclei frequency in buccal cells provided detailed discussions of design, methods, and results; potential confounders and other exposures that could pose a risk of genotoxicity were considered and excluded either in the design or data analysis. Associations with exposure duration also were observed by some researchers. Aglan (2018) analyzed micronuclei frequency in EBC from hair stylists who routinely conducted hair straightening treatments and compared them to a group of hair stylists who did not conduct these treatments. Formaldehyde concentrations can be high when hair straightening treatments are used, and 15-minute TWA concentrations greater than 1.9 mg/m^3 were measured in this group. An increase in MN frequency was observed between the referent group and exposed groups stratified by exposure duration (below or above 5 years). However, there is more uncertainty in these results because reporting deficiencies prevented analysis of the potential for selection bias. While Costa (2019) reported a nonsignificant increase across tertiles of formaldehyde concentration above 0.2 ppm among anatomy/ pathology workers, the authors did not observe a trend in the frequency of

nuclear buds across exposure duration from less than 8 years to over 14 years. Other studies of workers with mean exposure duration over 5 years also reported associations with exposure duration (Ladeira et al., 2011; Viegas et al., 2010).

Fewer studies are available that assessed micronuclei in nasal cells, but results were generally consistent. Significant differences in nasal micronuclei frequency were observed among anatomy students after an 8-week course (Ying et al., 1997), pathology workers compared to unexposed workers at the same institutions (Burgaz et al., 2001), and between formaldehyde production workers (Ye et al., 2005) or plywood production workers (Ballarin et al., 1992) compared to their referent groups. Formaldehyde concentrations among exposed groups averaged 0.1–>1.0 mg/m^3. One study did not observe formaldehyde-related changes in nasal cells of embalming students (Suruda et al., 1993), but did report an increase in micronuclei with acentric fragments (centromere negative micronuclei) using fluorescence in situ hybridization (FISH) (Titenko-Holland et al., 1996). These results suggest that the predominant damage in these cells consisted of DNA and/or chromosomal breaks.

Most of a large set of studies that measured micronuclei in peripheral blood lymphocytes reported increased levels among exposed participants working in diverse exposure settings and in several countries (Costa et al., 2019; Wang et al., 2019; Aglan and Mansour, 2018; Souza and Devi, 2014; Bouraoui et al., 2013; Costa et al., 2013; Costa et al., 2011; Ladeira et al., 2011; Jiang et al., 2010; Viegas et al., 2010; Costa et al., 2008; Orsiere et al., 2006; Ye et al., 2005; He et al., 1998; Suruda et al., 1993). Several of these studies included a large sample size, and all provided detailed discussions of design, methods, and results, including how potential confounders and other exposures that could pose a risk of genotoxicity were considered and excluded, either in the design or data analysis. Costa et al. (2019) reported that the frequency of micronuclei in PBL and EBC were correlated in their study population. A clear concentration-related response in micronucleus frequency measured in peripheral blood lymphocytes was reported among plywood production workers in two studies that evaluated effects across multiple exposure categories (Jiang et al., 2010; Ye et al., 2005). Micronuclei frequency (and centromeric micronuclei) increased with cumulative exposure (Wang et al., 2019; Suruda et al., 1993) and the duration of exposure (Aglan and Mansour, 2018; Souza and Devi, 2014; Bouraoui et al., 2013; Lin et al., 2013; Ladeira et al., 2011; Jiang et al., 2010; Viegas et al., 2010). Observed effects were independent of confounding by age, gender, or smoking status.

A study of anatomy students did not observe changes in micronuclei in peripheral blood lymphocytes after an 8-week course, although increased levels were observed in buccal and nasal cells, suggesting that changes in lymphocytes may occur after a longer duration of formaldehyde exposure (Ying et al., 1997). Lin et al. (2013) did not observe an increase in micronucleus frequency across formaldehyde exposure categories among plywood workers in China. However, the referent group was exposed to mean concentrations of 0.13 mg/m^3, a level associated with increased micronucleus frequency in another study of plywood workers (Jiang et al., 2010).

The sensitivity of the micronucleus assay can be enhanced by probing cells with pancentromeric DNA probes. A micronucleus that has a single centromere (C1 + MN) suggests chromosome migration impairment, and the presence of two or more centromeres (Cx + MN) indicates centromere amplification, with both conditions indicating aneuploidy (Iarmarcovai et al., 2006). Orsiere et al. (2006) and Bouraoui et al. (2013) evaluated micronuclei in lymphocytes using FISH and a pancentromeric probe and found increased levels of centromeric micronulei, including monocentromeric micronulei (C1 + MN) and multicentromeric micronuclei (Cx + MN) among exposed pathology and anatomy lab workers. The enhanced chromosome loss is consistent with the increase in aneuploidy in lymphocytes reported by Zhang et al. (2010).

DNA Damage

Most studies of DNA single-strand breaks, DNA crosslinks, apurinic or apyrimidinic sites, and sites with incomplete DNA repair using the Comet assay observed associations in peripheral blood leukocytes with occupational formaldehyde exposure involving workers in plywood or furniture manufacturing, use of melamine resin and pathology laboratories (Zendehdel et al., 2017; Costa et al., 2015; Peteffi et al., 2015; Lin et al., 2013; Gomaa et al., 2012; Costa et al., 2011; Jiang et al., 2010; Costa et al., 2008) (see Table-A24). A 1.5- to 3-fold difference was observed comparing exposed groups to their referent groups at average concentrations as low as 0.09 mg/m^3 (Zendehdel et al., 2017), 0.14 mg/m^3 (Jiang et al., 2010) or 0.04–0.11 mg/m^3 (Peteffi et al., 2015). A clear concentration-related response was observed in plywood plant workers (Lin et al., 2013; Jiang et al., 2010). In addition to the cross-sectional comparisons, an increased level of damage to DNA, indicated by increased tail moment levels in the Comet assay, was associated with formaldehyde exposure over an 8-hour work shift (Lin et al., 2013) and after an exposure for 4 hours/day for 5 days during a controlled human exposure study (Zeller et al., 2011). One study of workers in medium density fiberboard manufacture did not observe increases in Comet assay measures in the exposed group at a mean 8-hour TWA 0.25 ± 0.07 mg/m^3 (Aydın et al., 2013). The range of exposure levels (0.12–0.41 mg/m^3) was lower than most of the studies that evaluated DNA damage using the Comet assay, and almost half of the exposed workers in this study reported using personal protective equipment.

An increased level of DPXs was associated with formaldehyde exposure in a few studies, both across an 8-hour work shift (Lin et al., 2013), and in comparisons of formaldehyde-exposed workers and their referent groups (Shaham et al., 2003; Shaham et al., 1997). Lin et al. (2013) also compared DPX rates between formaldehyde-exposed plywood workers and a referent group but did not observe differences by exposure group. There was no trend across levels of exposure or duration of employment, possibly because the comparison group had significant exposure to formaldehyde (0.019–0.252 mg/m^3) and workers had been employed only for a mean of 2.5 years. Shaham et al. (2003) found higher DPX levels in peripheral lymphocytes among a group of pathologists with a mean duration of exposure of 16 years compared to administrative workers from the same hospitals. While DPX levels in the exposed group were comparable to the exposed

groups studied by Lin et al. (2013), DPX levels in the administrative workers were 60% less than those measured in the referent group of woodworkers, perhaps reflecting their lower formaldehyde exposure. Analyses ruled out potential confounding by age, gender, smoking, education, and country of origin. Shaham et al. (2003) also observed higher levels of pantropic p53 protein (mutant plus wild-type protein) in serum in the exposed group compared to unexposed, with a particularly strong association in males (pantropic p53 >150 pg/mL, adjusted OR = 2.0 (95% CI 0.9–4.4)). Increased serum pantropic p53 levels (p53 >150 pg/mL) was associated with mutant p53 content, and also with elevated DPX (OR = 2.5, 95% CI 1.2–5.4), suggesting a link between increases in DPX and overexpression of mutant p53 protein, an indication of loss of tumor suppressor gene capability.

Malondialdehyde-deoxyguanosine (M_1dG) adducts in DNA extracted from whole blood were elevated in pathologists who spent time conducting tissue fixation (mean formaldehyde 0.212 ± 0.047 mg/m^3) compared to workers and students in other science labs (Bono et al., 2010). The prevalence of M_1dG DNA adducts was increased in the entire group of pathologists compared to the referent group among whom average formaldehyde concentrations were 0.028 mg/m^3. Increased levels also were observed among a subgroup exposed to 0.07 mg/m^3 formaldehyde and higher. This finding suggests the presence of formaldehyde-associated DNA damage concurrent with the induction of oxidative stress. An increase in oxidative stress, indicated by elevated plasma levels of malondialdehyde (MDA), was observed among employees at a cosmetic manufacturing company, who also had higher plasma levels of p53 compared to a group of employees in a hospital administrative department with no formaldehyde exposure (Attia et al., 2014). Although no air monitoring was conducted, the cosmetics workers had higher urinary formate levels compared to the referent group. Both plasma MDA and plasma p53 levels were related to urinary formate levels and also to each other. Regression analyses were adjusted for age and gender. Together, these two studies suggest that formaldehyde may increase systemic oxidative stress, which may be related to observed increases in peripherial white blood cell genotoxicity.

DNA Repair Protein Activity

O^6-alkylguanine DNA alkyl-transferase activity in peripheral blood lymphocytes of students after 9 weeks or 3-months exposure to formaldehyde in embalming or anatomy labs was compared to enzyme activity prior to the beginning of the courses. Although an association with decreased activity was indicated in one study of embalming students (Hayes et al., 1997), this finding was not confirmed by a subsequent study of anatomy students (Schlink et al., 1999).

Susceptibility: Gene-Environment Interaction

A few studies of genotoxicity among formaldehyde-exposed groups also evaluated differences in subgroups defined by polymorphic variants in genes coding for proteins involved in the detoxification of xenobiotic toxic substances, including glutathione-S-tranferases (GSTM1, GSTT1, GSTP1), CYP2E1, and specifically, formaldehyde (alcohol dehydrogenase (ADH5) (see Table

A-24). Polymorphisms in DNA repair proteins also were studied includingthe X-ray repair cross-complementing genes (XRCC1, XRCC2, XRCC3), RAD51, PARP1, and MUTYH. This included genes of Fanconi anemia pathway (FANCA, BRIP1). The frequency of chromosomal aberrations in lymphocytes was higher in a formaldehyde-exposed group but did not vary by GSTT or GSTM polymorphism (Santovito et al., 2011). However, the GSTM1 null variant and the GSTP1 codon 105 Val allele was associated with an increased olive tail moment and MN frequency, respectively, among exposed individuals, but not in the referent group (Jiang et al., 2010). Costa et al. (2015) and Costa et al. (2019) also reported an increase in MN frequency in exfoliated buccal cells among exposed individuals with the Val variant in the GSTP1 rs1695 polymorphism, whereas chromosomal aberrations (CSAs) were more prevalent among the exposed group homozygous for the Ile allele. This research group also reported an increase in nuclear buds in buccal cells among exposed individuals with the A variant in the CYP2E1 rs6413432 polymorphism while exposed individuals homozygous for the wildtype T allele had a higher % tDNA measured in the comet assay. These associations were not observed in the referent group. In addition, the variant allele for the ADH5 Val309Ile polymorphism was associated with an increased frequency of micronuclei in lymphocytes among exposed individuals, but not in the referent group (Ladeira et al., 2013). The frequency of nuclear buds was associated with formaldehyde exposure and among carriers of the XRCC3 *Met* variant allele in both exposed and referent individuals, but effect modification was not apparent (Ladeira et al., 2013). Costa et al. (2019) did not observe associations with the XRCC gene polymorphisms and micronuclei frequency in EBC or PBL among formaldehyde exposed workers. However, micronuclei frequency was increased in PBL among exposed individuals with the Ala variant in the FANCA rs719823 variant. Therefore, genetic differences may alter susceptibility to the cytogenetic effects of formaldehyde, but more definitive research is needed.

Table A-24. Summary of genotoxicity of formaldehyde in human studies

Reference and study design	Exposure	Results
Chromosomal Damage and Induction of DNA repair		
Prevalence Studies		
Costa et al. (2015) Portugal Prevalence study **Population:** 84 anatomy pathology workers from 9 hospital laboratories, exposed to formaldehyde for at least 1 year, compared to 87 unexposed employees from administrative	Exposure assessed via air sampling and deriving an 8-hr TWA for each subject. Exposure concentration: Mean: 0.38 ppm (0.47 mg/m^3) Range: 0.28–0.85 ppm (0.34–1.05 mg/m^3)	Comparison of exposed (*N*=84) and referent (*N*=87), frequencies of chromosome aberrations (CA), structural and numerical Aberration / MR[a] / 95% CI Total CA 1.91 1.44–2.53 CSAs 2.07 1.27–3.38 CTAs 1.86 1.39–2.48 Gaps 1.65 1.34–2.03 Aneuploidies 1.64 1.36–1.98 Aberrant cells 1.66 1.28–2.17

Reference and study design	Exposure	Results
offices in same geographic area. Exclusions: cancer history, radiation therapy or chemotherapy, surgery with anesthesia or blood transfusion in last year. Exposed and referent similar for mean age 39 years, 77% females, 25% smokers. **Outcome:** Peripheral blood samples, coded, analyses blinded to exposure status. Chromosome aberrations structural and numerical), duplicates cultured 51 hours (cited cited Roma-Torres et al., 2006), 4% Giemsa stain; scored 100 metaphases per person, CTAs & CSAs according to Savage et al. (1976); gaps not included. Exposed compared to unexposed using Mann-Whitney U-test for CA measures; negative binomial regression for untransformed total-CAs, CSAs, CTAs, gaps, aneuploidies, & aberrant cells; Poisson regression for untransformed multiaberrant cells.	Exposure duration 12.0 ± 8.2 yrs	Multi-aberrant cells 3.96 2.09−7.48 [a] MR − mean ratio; all models adjusted for age, gender and smoking habit, multi-aberrant cells MR also adjusted for fruit consumption (# pieces eaten per day) No associations observed for models of formaldehyde exposure as continuous variable, exposure duration or professional activity on genotoxicity endpoints (data not provided by authors) **Mean SCE per cell in peripheral lymphocytes: ratio of exposed to referent** SCE/cell: Ratio 1.27; 95% CI 1.10 −1.46 Poisson regression adjusted for gender, smoking, and age.
Lan et al. (2015) China Prevalence study **Population:** 43 formaldehyde-melamine workers (95% employed for >1 yr) compared to 51 workers from other regional factories no formaldehyde exposure frequency-matched by age and gender; participation rates exposed 92%, referent 95%; selected	Personal monitors for 3 d over entire shift within a 3-wk period. Formaldehyde concentration: 8 h TWA Exposed Median: 1.38 ppm (1.7 mg/m^3) 10th & 90th percentile: 0.78, 2.61 ppm (0.96, 3.2 mg/m^3)	Among all 24 chromosomes analyzed, elevated IRR for monosomy found for chromosomes 1, 5, 7, 4, 19, 10, 16, 21, 2, 8, 18, 12, 20, 13, 6, and 14 ($p < 0.05$, Table 2 in Lan et al.); elevated IRR for trisomy found for chromosomes 5, 19, 21, 1, 20, and 16; elevated IRR for tetrasomy found for chromosomes 4, 15, 17, 14, 3, 18, 8, 12, 2, 10, and 6. **Selected Comparison of Chromosome Aberration Rates*** **Chromosome** / **IRR** / **95% CI** / ***p*-Value** *Monosomy* 1 2.31 1.61−3.31 6.02E-06 5 2.24 1.57−3.20 9.01E-06

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr><td>subset with scorable metaphases, high formaldehyde levels among exposed, comparable referents with scorable metaphases (29 exposed and 23 referent).
Outcome: Chromosome-wide aneuploidy in CFU-GM colony cells cultured for 14 d using OctoChrome FISH; scored minimum 150 cells/ subject; analysis blinded to exposure. Analyzed using negative binomial regression controlling for age and gender; incidence rate ratio (IRR). Also evaluated potential confounding from current smoking and alcohol use, recent infections, current medication use, and body mass index (Supplemental tables in the paper)
Related reference: Zhang et al. (2010)</td><td>Referent
0.026 ppm (0.032 mg/m^3)
10th & 90th percentile: 0.015, 0.026 ppm (0.019, 0.032 mg/m^3)

Formaldehyde LOD: 0.012 ppm

Personal sampling for organic compounds on 2 or more occasions. No chloroform, methylene chloride, tetra-chloroethylene, trichloro-ethylene, benzene, or hydrocarbons were detected; urinary benzene at background levels and similar between groups</td><td>
<table>
<tr><td>7</td><td>2.17</td><td>1.53−3.08</td><td>1.57E-05</td></tr>
<tr><td>4</td><td>2.02</td><td>1.40−2.90</td><td>0.00015</td></tr>
<tr><td>19</td><td>1.74</td><td>1.29−2.34</td><td>0.00026</td></tr>
<tr><td>10</td><td>1.86</td><td>1.30−2.65</td><td>0.00064</td></tr>
<tr><td>16</td><td>1.54</td><td>1.12−2.12</td><td>0.0075</td></tr>
<tr><td>*Trisomy*</td><td></td><td></td><td></td></tr>
<tr><td>5</td><td>3.40</td><td>1.94−5.97</td><td>1.98E-05</td></tr>
<tr><td>19</td><td>2.07</td><td>1.24−3.46</td><td>0.0055</td></tr>
<tr><td>21</td><td>2.09</td><td>1.22−3.57</td><td>0.0071</td></tr>
<tr><td>*Tetrasomy*</td><td></td><td></td><td></td></tr>
<tr><td>4</td><td>1.64</td><td>1.21−2.21</td><td>0.0012</td></tr>
<tr><td>15</td><td>3.10</td><td>1.53−6.28</td><td>0.0017</td></tr>
<tr><td>17</td><td>2.40</td><td>1.33−4.32</td><td>0.0036</td></tr>
</table>
* Chromosomes with IRR with *p*-values < 0.001.

Increased frequency of structural chromosome aberrations in chromosome 5, IRR 4.15, 95% CI 1.20−14.35 (*p* = 0.024).</td></tr>
<tr><td>Santovito et al. (2014)
Italy
Prevalence study
Population: 20 female nurses from 2 analogous departments in 2 hospitals (mean age 37 yr) ; 20 unexposed from administrative departments of same hospital (mean age 39.6 yr); all nonsmokers and did not consume alcohol
Outcome: Peripheral blood samples, coded. Cultures incubated for 48 hr for CA and 72 hr for SCE; CA slides stained with 5% Giemsa, scored 200 metaphases per subject,</td><td>All exposed used protective equipment; no formaldehyde measurements; nurses also exposed to antibiotics, cytostatic drugs, anesthetics and sterilants

Employment duration: Exposed 11.8 yr, range 1−28 yr; Referent 11.2 yr, range 7−20 yr</td><td>**Frequency of Chromosomal Aberrations and SCEs among nurses and referent (mean ± SE)**
<table>
<tr><th></th><th>#</th><th>Nurses</th><th>Referent</th></tr>
<tr><td>CA/ NSM</td><td>20</td><td>0.025 ± 0.003</td><td>0.02 ± 0.003</td></tr>
<tr><td>Cells with aberrations/ NSM</td><td>20</td><td>0.025 ± 0.003</td><td>0.02 ± 0.003</td></tr>
<tr><td>SCEs/ NSM</td><td>20</td><td>6.55 ± 0.033*</td><td>4.10 ± 0.37</td></tr>
</table>
NSM: number of scored metaphases
*p <0.001

No association CAs or SCEs with age or duration</td></tr>
</table>

Reference and study design	Exposure	Results
SCE 50 metaphases scored per subject; Mean frequencies compared, Wilcoxon test		
Costa et al. (2013) Portugal Prevalence study **Population:** 35 pathology workers from 4 hospital laboratories, exposed to formaldehyde for at least 1 yr (88.6% female, mean age 41.2 yr, 20% smokers), compared to 35 unexposed employees from same work area (80% female, mean age 39.8 yr, 20% smokers). **Outcome:** SCE, coding and analysis blinded; stain fluorescence plus Giemsa, scored 50 M_2 metaphases/ subject by one reader Related references: Costa et al. (2011); Costa et al. (2008)	Exposure assessed via air sampling and deriving an 8-hr TWA for each subject. Exposure concentration: Mean: 0.44 mg/m^3 Range: (0.28–0.85) mg/m^3 Exposure duration 12.5 (1–30) yrs	Mean SCE per cell 1.3-fold higher in exposed workers compared to controls (p <0.05, Student's t-test). Univariate analyses presented in Figure 1 of Costa et al. (2013) **Mean SCE per cell in peripheral lymphocytes: ratio of exposed to referent** SCE/cell: Ratio 1.245; 95% CI 0.594 –1.897 Multivariate analysis adjusted for gender, smoking, and age
Musak et al. (2013) Slovakia Prevalence study **Population:** 105 technicians and pathologists at hospital labs (79% female, mean age 41.7 yrs, 27.6% smokers) compared to 250 other medical staff (89% female, mean age 36.2 yrs, 19.2% smokers), all healthy. **Outcome:** Differences in frequency of chromosomal aberration in peripheral blood lymphocytes, blinded analysis, 100 mitoses scored/ subject, 2 scorers	Air monitoring once per year (no details provided). Exposure conc.: Mean: 0.32 mg/m^3 Range: 0.14–0.66 mg/m^3 Exposure duration: Mean: 14.7 ± 10.4 yrs Range: NR	**Chromosome aberrations in peripheral lymphocytes** CA: OR 1.70; 95% CI 1.6–2.72 CTA: OR 1.37; 95% CI 0.85–2.19 CSA: OR 1.57; 95% CI 0.98–2.53 Chromosomal exchange: OR 2.6; 95% CI 1.1–5.9 Binary logistic regression controlling for age, gender, job type, and smoking

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr>
<td>Gomaa et al. (2012)
Egypt
Prevalence study
Population: 30 workers in pathology, histology and anatomy laboratories at a university (30% female, mean age 42.5 yr) compared to 15 referents (46.7% female, mean age 39.3 yr). Source of referent was not described.
Outcome: Chromosome aberrations in peripheral blood lymphocytes, cultured 72 hr, blinding not described; mean # per 100 metaphases; Difference between exposed and referent, Student's t-test</td>
<td>No formaldehyde measurements; exposure defined by job type

Mean employment duration 14.3 yr</td>
<td>Chromosomal aberrations in peripheral lymphocytes
<table>
<tr><th>Structural</th><th>Referent</th><th>Exposed</th></tr>
<tr><td>Chromatid gap & break</td><td>1.9 ± 0.36</td><td>6.5 ± 0.65*</td></tr>
<tr><td>Chromatid deletion</td><td>8.7 ± 0.55</td><td>15.5 ± 0.47*</td></tr>
<tr><td>Ring chromosome</td><td>5.5 ± 0.33</td><td>16.4 ± 0.29*</td></tr>
<tr><td>Dicentric chromosome</td><td>0.9 ± 0.41</td><td>9.0 ± 0.54*</td></tr>
<tr><td>Total</td><td>20.0 ± 0.27</td><td>46.4 ± 0.35</td></tr>
<tr><td>Numerical</td><td></td><td></td></tr>
<tr><td>Aneuploidy</td><td>0.2 ± 0.12</td><td>0.7 ± 0.10</td></tr>
<tr><td>Polyploidy</td><td>0.6 ± 0.14</td><td>0.9 ± 0.09</td></tr>
</table>
* Student's t-test, p <0.05; mean per 100 metaphases ± SE

No association with age or gender, ANOVA</td>
</tr>
<tr>
<td>Santovito et al. (2011)
Italy
Prevalence study
Population: 20 pathology workers (70% female, mean age 45.7 yr) compared to 16 workers from the same hospital (43.8% female, mean age 42.1 yr). All subjects were non-smokers and had not consumed alcohol in 1 yr.
Outcome: Frequency of chromosome aberrations per cell and mean % cells with aberrations; Venous blood sample collected at end of shift on same day as formaldehyde measurements, samples coded and processed within 4 hrs of collection, cells harvested 48 hr, 5% Giemsa stain, scored 100 metaphases/subject</td>
<td>Exposure conc:
Personal air sampling, 8-hr duration.
Referent: Mean: 0.036 ± 0.002 mg/m^3
Pathologists: Mean: 0.073 ± 0.013 mg/m^3
LOD 0.05 mg/mL

Exposure duration:
Mean: 13 yrs
Range: 2–27 yrs</td>
<td>Chromosomal aberrations in peripheral lymphocytes
<table>
<tr><th></th><th>Referent</th><th>Exposed</th></tr>
<tr><td>Mean CA/cell</td><td>0.011 ± 0.004</td><td>0.03 ± 0.004*</td></tr>
<tr><td>% of cells with aberrations</td><td>1.00 ± 0.342</td><td>2.50 ± 0.286</td></tr>
</table>
*p <0.001, Mann-Whitney U test

Effects of exposure on chromosomal aberrations and cells with aberrations (coefficient (SE))
<table>
<tr><th></th><th>Exposure</th><th>p- Value</th></tr>
<tr><td># CA</td><td>0.960 (0.275)</td><td>0.001</td></tr>
<tr><td># cell with aberrations</td><td>0.838 (0.287)</td><td>0.004</td></tr>
</table>
Generalized linear models with Poisson error distribution, adjusted for age</td>
</tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr><td>Jakab et al. (2010)
Hungary
Prevalence study
Population: 37 female workers in 3 hospitals & 1 university pathology department (21 exposed to formaldehyde alone (mean age 43.3 yr, 23.8% smokers), compared to 37 healthy female unexposed health-service staff (mean age 41.8 yr, 16.2% smokers).
Outcome: Peripheral lymphocytes; CA, SCE, premature centromere division (PCD), mitoses with >3 chromosomes with PCD (centromere separation general (CSG)), CA stain 5% Giemsa, cells harvested 50 hr, scored 100 metaphases/ subject. SCE fluorescence plus Giemsa; scored 50 cells/ subject; analyses blinded</td><td>Exposure assessed via records on area air samples, measured within 1–3 yrs of data collection.

Exposure Concentration:
8-hr TWA: 0.9 mg/m^3
Range: 0.23–1.21 mg/m^3
Exposure duration:
Mean: 17.7 yrs
Range: 4-34 yrs</td><td>Cytogenetic analysis in cultured peripheral lymphocytes (mean ± SD)
<table><tr><th></th><th>Unexposed</th><th>Exposed</th></tr><tr><td>Total CA</td><td>1.62 ± 0.26</td><td>3.05 ± 0.62*</td></tr><tr><td>Chromatid-type aberrations</td><td>1.00 ± 0.20</td><td>2.35 ± 0.46*</td></tr><tr><td>Chromosome-type aberrations</td><td>0.62 ± 0.18</td><td>0.70 ± 0.26</td></tr><tr><td>Aneuploidy</td><td>8.89 ± 0.66</td><td>5.4 ± 0.61*</td></tr><tr><td>SCE (%/cell)</td><td>6.16 ± 0.16</td><td>6.36 ± 0.26</td></tr><tr><td>High frequency SCE</td><td>3.76 ± 1.14</td><td>7.05 ± 2.19</td></tr><tr><td>PCD (%)</td><td>7.6 ± 0.84</td><td>13.65 ± 1.59*</td></tr><tr><td>PCD (CSG)</td><td>5.57 ± 0.66</td><td>8.8 ± 1.07*</td></tr></table>*p <0.05, Student's t-test, compared to controls
SCE % and mean HF/SCE higher in referent and exposed smokers; mean SCE % associated with older age</td></tr>
<tr><td>Zhang et al. (2010)
China
Prevalence study
Population: 43 formaldehyde-melamine workers (95% employed for >1 yr) compared to 51 workers from other regional factories frequency-matched by age and gender; participation rates exposed 92%, referent 95%; Analyzed subset of exposed (n=10, 9 male, 1 female, mean age 31 yr) and referent (n =12, 11 male, 1 female, mean age 32 yr)
Outcome: Chromosome aberration in peripheral blood cells, blinded to</td><td>Personal monitors for 3 d within a 3-wk period.
Formaldehyde concentration: 8 h TWA
Exposed
Median: 1.57 mg/m^3
10th & 90th percentile: 0.74, 3.08 mg/m^3

Referent
0.039 mg/m^3
10th & 90th percentile: 0.022, 0.039</td><td>Leukemia-specific chromosome changes:

Significant increase chromosome aneuploidy in cultured CFU-GM colony cells among subset of high exposed (n =10) compared to matched controls (n = 12)
Data provided in Figure 4 of Zhang et al. (2010).

Analyzed using negative binomial regression (exposed compared to unexposed) controlling for age, gender, and smoking

Mundt et al. presented individual data in graphs for chromosome 7 and chromosome 8 (n = 10 exposed and n = 12 controls), noting smoking status and whether 150 or more cells were evaluated. No patterns apparent.</td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr><td>exposure. Chromosome aneuploidy in cultured CFU-GM colony cells using FISH; monosomy 7 and Trisomy 8; scored minimum 150 cells/ subject.
Related reference:
Mundt et al. (2017); Lan et al. (2015); Gentry et al. (2013)</td><td></td><td></td></tr>
<tr><td>Costa et al. (2008)
Portugal
Prevalence study
Population: 30 pathology lab workers (4 hospitals), (70% female, mean age 38 yr, 27% smokers) compared to 30 administrative employees matched by age, gender, lifestyle, smoking habits and work area (63.3% female, mean age 37 yrs, 23% smokers).
Outcome: Peripheral lymphocytes; blood samples collected 10−11 am; processed immediately; stain fluorescence plus 5% Giemsa, SCE/ cell 50 s division metaphases scored by one observer, Scored blind to exposure status. Effect of smoking and gender also analyzed</td><td>Exposure assessed via air sampling at breathing zone and deriving an 8-hr TWA for each subject

Concentration:
Mean: 0.54 mg/m^3
Range: (0.05−1.94) mg/m^3

Duration: 11 yrs
Range: (0.5−27) yrs</td><td>Mean SCE per cell in peripheral lymphocytes
<table><tr><th></th><th>Controls</th><th>Exposed</th></tr><tr><td>SCE/ cell</td><td>4.49 ± 0.16</td><td>6.13 ± 0.29*</td></tr></table>*p <0.05, Student's t-test

No association of SCE with gender or age. Smoking increased SCE among referent group (smoking prevalence 23% in referent, 27% in exposed.

No association of SCE with duration of exposure</td></tr>
<tr><td>Pala et al. (2008) Italy
Prevalence study
Population: 36 lab workers (66.7% female, mean age 40.1 yr, 16.7% smokers)
Outcome: CA and SCE, in peripheral lymphocytes (blood sampled at end of</td><td>Personal air monitoring (8-hr sample)
High exposure group: ≥ 0.026 mg/m^3, 75th percentile (range 0.005−0.269 mg/m^3) and low-exposure group: <0.026 mg/m^3
Concentration:</td><td>Frequency chromosome aberrations in peripheral lymphocytes
<table><tr><th></th><th>CA</th><th>SCE</th></tr><tr><td>< 0.026 mg/m^3</td><td>2.95 ± 1.79 (n=19)</td><td>6.57 ± 1.38 (n=17)</td></tr><tr><td>≥ 0.026 mg/m^3</td><td>2.22 ± 1.27 (n=5)</td><td>5.06 ± 0.76 (n=2)</td></tr><tr><td>Means ratio (95% CI)</td><td>0.83 (0.42−1.64)</td><td>0.81 (0.56−1.18)</td></tr></table></td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr><td>8-hour) Blinded analyses, CA: cells harvested at 48 hr, 100 metaphases/ subject, SCE: harvest at 72 hr, 30 2nd division cells/ subject.</td><td>Low (n = 27): 0.015 (0.005−0.0254) mg/m^3
High (n = 9): 0.056 (0.026−0.269) mg/m^3</td><td>Multivariate regression models adjusting for gender, age and smoking; Poisson model for CA, SCE log-normal random effects model
Authors did not use a referent group</td></tr>
<tr><td>Ye et al. (2005) China
Population: 18 workers at a formaldehyde plant at least 1 yr (38.9% female, mean age 29 yr, , and 16 workers exposed to indoor air formaldehyde via building materials (75% female, mean age 22 yr) compared to 23 students with no known source of formaldehyde exposure (dormitories) (48% female, mean age 19 yr); all nonsmokers
Outcome: SCE in peripheral lymphocytes, time of sample not stated; stain Giemsa solution, analysis blinded, 30 M_2 lymphocytes analyzed/ subject.</td><td>Area samples;
Exposure duration:
Workers 8.5 (1−15) yrs
Waiters 12 wks

TWA Concentration
Controls
0.011 ± 0.0025 mg/m^3
Max. 0.015 mg/m^3
Wait staff
0.107 ± 0.067 mg/m^3
Max. 0.30 mg/m^3
Workers
0.985 ± 0.286 mg/m^3
Max. 1.694 mg/m^3</td><td>SCE frequency by exposure group
<table><tr><th></th><th>Referent</th><th>Wait Staff</th><th>Formaldehyde workers</th></tr><tr><td>Mean SCE</td><td>6.38 ± 0.41</td><td>6.25</td><td>8.24 ± 0.89*</td></tr></table>*p <0.05, ANOVA. Values estimated from graph in Figure 2 of Ye et al.</td></tr>
<tr><td>(Shaham et al., 2002) Israel
Prevalence study
Population: 90 workers from 14 hospital pathology departments (65 females, 25 males; mean age 44.2 yr, 34% smokers) compared to 52 administrative workers from the same hospitals (8 females, 44 males; mean age 41.7 yr, 46.9% active smokers, 53.1% nonsmokers)
Outcome: SCE in peripheral lymphocytes; Mean # SCEs per chromosome and proportion of high</td><td>Personal and area samples, sampling at different points in work day, sampling duration averaged 15 min
Exposure concentration:
Low level exposure:
Mean: 0.49 mg/m^3
Range: 0.05−0.86 mg/m^3

High level exposure:
Mean: 2.76 mg/m^3
Range: 0.89−6.89 mg/m^3

Exposure duration:
Mean: 15.4 yrs</td><td>SCE frequency in peripheral lymphocytes by exposure group and smoking status (mean ± SE)
<table><tr><th></th><th>Mean number SCEs per chromosome</th><th>Mean proportion of high frequency cells</th></tr><tr><td>Unexposed</td><td>0.19 ± 0.004</td><td>0.44 ± 0.02</td></tr><tr><td>Exposed</td><td>0.27 ± 0.003*</td><td>0.88 ± 0.01*</td></tr><tr><td>No smoking</td><td></td><td></td></tr><tr><td>Low</td><td>0.28 ± 0.004</td><td>0.88 ± 0.015</td></tr><tr><td>High</td><td>0.26 ± 0.021</td><td>0.86 ± 0.016</td></tr><tr><td>Smoking</td><td></td><td></td></tr><tr><td>Low</td><td>0.27 ± 0.007</td><td>0.89 ± 0.018</td></tr><tr><td>High</td><td>0.28 ± 0.006</td><td>0.92 ± 0.021</td></tr></table>*p <0.01, ANOVA adjusting for age, gender, smoking status, education years and origin (ethnicity)

No association with exposure duration (≤15 years and >15 years) with adjustment for age, gender, smoking status, education years and origin (ethnicity)</td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr><td>frequency cells compared between exposed and referent. High frequency cells defined as > 8 SCEs; blinding not described, stain fluorescence plus 5% Giemsa, scored 30–32 cells/ subject.
Related references:
Shaham et al. (1997)</td><td>Range: 1–39 yrs</td><td></td></tr>
<tr><td>Lazutka et al. (1999)
Lithuania
Prevalence study
Population: Carpet and plastic manufacturing; Carpet plant, exposed, 38 male, 41 female (age 22–65 yr, 49% smokers); unexposed, 64 male, 26 female, 30% smokers; Plastic plant, exposed 34 male, 63 female (age 28–64 yr, 37% smokers); unexposed 64 males, 26 females
Outcome: CA in peripheral blood lymphocytes; fluorescence plus Giemsa stain, cells harvested 72 hr, scored 100 metaphases/ subject on coded slides.</td><td>Industrial hygiene area measurements reported by plants; carpet plant, formaldehyde 0.3–1.2 mg/m^3, styrene 0.13–1.4 mg/m^3, phenol 0.3 mg/m^3; plasticware plant, formaldehyde 0.5–0.9 mg/m^3, styrene 4.4–6.2 mg/m^3, phenol 0.5–0.75 mg/m^3

Duration exposure, carpet plant: 2 mo-21 yr; plastic plant: 2 mo–25 yr</td><td>Frequency of chromosomal aberrations in peripheral blood lymphocytes by exposure (CA/ 100 cells ± SEM)
<table><tr><th></th><th>#</th><th>CA Frequency</th></tr><tr><td>Carpet Workers</td><td></td><td></td></tr><tr><td>Exposed</td><td>79</td><td>3.79 ± 0.32*</td></tr><tr><td>Referent</td><td>90</td><td>1.68 ± 0.13</td></tr><tr><td>Plasticware workers</td><td></td><td></td></tr><tr><td>Exposed</td><td>97</td><td>4.17 ± 0.29*</td></tr><tr><td>Referent</td><td>90</td><td>1.68 ± 0.13</td></tr></table>*p < 0.0001; ANOVA adjusted for age
Predominant types of damage were chromatid and chromosome breaks

Duration of exposure not associated with CA frequency; Age and smoking (data not shown) were not associated with CA frequency</td></tr>
<tr><td>Shaham et al. (1997)
Israel
Prevalence study
Population: 13 pathology workers (mean age 42 yr, 23% smokers) compared to 20 referent workers matched by age (mean age 39 yr, 30% smokers).
Outcome: SCE in peripheral lymphocytes, Mean # per chromosome, stain fluorescence plus 5% Giemsa, blinding not described, mean of 30 cells/ individual,</td><td>Field and personal air sampling, sample duration 15 min, multiple times during work-day (# not reported).
Concentration:
Mean: not reported
Range: 1.7–1.97 mg/m^3
Personal samples:
Range: 3.4–3.8 mg/m^3

Exposure duration mean 13 yrs (range 2–25 yrs)</td><td>SCE (mean # per chromosome) in peripheral lymphocytes
<table><tr><th></th><th>Unexposed</th><th>Exposed</th></tr><tr><td>SCE</td><td>0.186 ± 0.035</td><td>0.22 ± 0.039*</td></tr></table>*p = 0.05, ANOVA adjusted for smoking status

years of exposure linearly correlated with mean number of SCE per chromosome, adjusting for smoking</td></tr>
</table>

Reference and study design	Exposure	Results
Related references Shaham et al. (1996)		
Kitaeva et al. (1996) Russia (translated) Prevalence study **Population:** 15 formaldehyde production workers (5 females, 10 males, mean age 38 yr), anatomy instructors (6 female, 2 male), mean age 41 yr) compared to 6 unexposed (mean age 28.5 yr) **Outcome:** Blood collection in 1988. CA: cells harvested at 72 hr; blinding not described. Unclear if statistical analyses were performed.	No quantitative exposure assessment Exposure duration: Formaldehyde production 9.7 yrs Anatomy instructors 17 yrs	**CA (% aberrant metaphases) in peripheral lymphocytes** % of metaphases at 72 hrs lymphocyte culture: Referent (n=6) 1.8 ± 0.6 (547 metaphases examined); Exposed Workers (n=8) 5.4 ± 1.9 (148 metaphases examined) No metaphases observed at 72 hours in lymphocyte cultures from anatomy instructors Authors reported that % CA was not dependent on age, gender and length of employment
Vasudeva and Anand (1996) India Prevalence study **Population:** 30 female medical students exposed 15 mos, compared to 30 age-matched nonmedical students. All 17–19 yrs old **Outcome:** chromosomal aberrations in peripheral blood samples, mean frequency aberrant metaphases, cells harvested at 72 hr, 100 cells/ subject; blinding not reported.	Exposure not quantified Exposure conc.: < 1.23 mg/m^3 Exposure duration: 15 mos	No significant difference in chromosomal aberrations between groups (p >0.5). Mean frequency of aberrant metaphases Exposed: 1.2% Unexposed: 0.9% *No additional quantitative information available*
Vargová et al. (1992) Czechoslovakia Prevalence study **Population:** 20 wood workers with at least 5 years of exposure (10 females, 10 males, mean age 42.3 yr), compared to 19 workers from the same	Task-based air sampling in breathing zone over 8 hours Exposure conc.: Range: 0.55−10.36 mg/m^3 Exposure duration: 5−>16 yrs	**Frequency of chromosomal aberrations in peripheral lymphocytes by exposure group** % aberrant cells: Exposed 3.08; Unexposed[a] 3.60 # breaks per cell[a]: Exposed 0.045; Unexposed[a] 0.030 [a] According to authors, both groups reported % aberrant cell levels above normal range (1.2−2%)

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr><td>plant with no known occupational contact with chemicals.
Outcome: CA frequency, peripheral lymphocytes, Giemsa staining, cells harvested 48 hr, 100 cells/ subject. Blinding not described.</td><td></td><td></td></tr>
<tr><td>Bauchinger and Schmid (1985)
Germany
Prevalence study
Population: 20 male paper makers exposed for at least 2 yrs (mean age 40.8 yr, 30% smokers) compared to 20 unexposed male workers from the same factory
Outcome: Peripheral lymphocytes, CA/ cell (scored 500 cells/ subject), cells harvested 48 hr, Giemsa staining; SCE/ cell (scored 50/ subject) analyzed using coded slides**,** SCE stratified by smoking status.</td><td>Exposure assessment based on air monitoring and job-function.
Exposure concentration.: ≈1.47 mg/m^3, plus 3.7 mg/m^3 for 45 min (supervisors) or 90 minutes (operators) per 8 hrs
Exposure duration
Mean: 14.5 yrs
Range: 2−30 yrs</td><td>**Frequency of CA and SCE/cell (mean ± SE) in peripheral lymphocytes**
<table><tr><th></th><th>Referent</th><th>Exposed</th></tr><tr><td>% cell with CA</td><td>0.86 ± 0.10</td><td>0.87 ± 0.08</td></tr><tr><td>SCE/ cell</td><td>9.53 ± .0.35</td><td>8.87 ± 0.24</td></tr><tr><td>*Aberrations/ cell*</td><td></td><td></td></tr><tr><td>Chromatid</td><td>0.0038 ± 0.0005</td><td>0.0042 ± 0.0005</td></tr><tr><td>Acentric fragments</td><td>0.0046 ± 0.0006</td><td>0.0034 ± 0.0005</td></tr><tr><td>Dicentrics</td><td>0.0005 ± 0.0002</td><td>0.0013 ± 0.0003*</td></tr><tr><td>Centric rings</td><td>0.0001 ± 0.0001</td><td>0.0003 ± 0.0001*</td></tr></table>*p <0.05, Mann-Whitney rank U test
Frequency of SCE was not associated with exposure when stratified by smoking</td></tr>
<tr><td>Thomson et al. (1984)
Great Britain
Prevalence study
Population: 6 pathology workers (2 female, 4 male, mean age 33.5 yr) compared to 5 referents (3 female, 2 male, mean age 27.8 yr) (study details on referent not provided)

Outcome: CA frequency, stain fluorescence plus Giemsa technique (Perry and Wolff, 1974), cells harvested 48 hr, slides coded and scored 100 1st division metaphases/</td><td>Personal air monitoring over 1−3 months before blood samples
Exposure conc.: TWA
Mean: 2.26 mg/m^3
Range: 1.14−6.93 mg/m^3
Exposure duration: 4–11 yrs, 2−4 hr/d, 2–3 d/wk</td><td>No significant difference in incidence of chromosome aberrations or SCE frequency found between groups.

SCE frequency (mean per cell)
Exposed (*N*=6) 6.78 ± 0.31
Referent (*N*=5) 6.44 ± 0.38

(individual data reported, analytic methods were not described)</td></tr>
</table>

Reference and study design	Exposure	Results
subject; SCE frequency, cells harvested 72 hr, 50 cells/ subject		
Fleig et al. (1982) Germany Prevalence study **Population:** 15 formaldehyde-manufacturing workers (mean age 50 yr) compared to 15 age-and gender matched unexposed workers from same plant. **Outcome:** Chromosome aberrations in peripheral blood lymphocytes cells harvested 70–72 hrs, 10% Giemsa stain; slides coded; scored 100 metaphases/ subject.	Personal air sampling. 1946−1971: <6.15 mg/m^3 (MAK) 1971−1982: <1.23 mg/m^3 (MAK) Duration: Mean: 28 yrs Range: 23−35 yrs	**Chromosomal aberrations in peripheral blood lymphocytes** Unexposed / Exposed Mean % aberrant cells including gaps: 3.33 / 3.07 Mean % aberrant cells excluding gaps: 1.07 / 1.67 *P* >0.05, Fisher's exact text Smoking habit not associated with CA (data not reported)
Suskov and Sazonova (1982) Russia Prevalence study **Population:** 31 phenol-formaldehyde workers (mean age 39.1 yr) compared to 74 referents matched by gender, smoking, alcohol consumption, and medication **Outcome:** Chromosomal aberrations via mean frequency of aberrant metaphases, Buckton and Evans (1973) method; cells harvested at 50 hr	Workers exposed to both phenol and FA. Area samples Exposure conc.: Formaldehyde Mean: 0.5 mg/m^3 Phenol mean: 0.3 mg/m^3 Exposure duration: 4 mos to 30 yrs	**Frequency of chromosomal aberrations by exposure group** Mean % aberrant cells / Referent / Exposed Aberrant metaphases: 2.4 ± 0.22 / 5.0 ± 0.40* Aberrant chromosomes per cell: 0.024 ± 0.002 / 0.058 ± 0.006* Chromosomal breaks per aberrant chromosome: 1.26 ± 0.076 / 1.27 ± 0.044 **p* <0.001, chi-square
Short-term Studies		
Ying et al. (1999) China **Population:** 23 nonsmoking anatomy	Air sampling, estimated TWA and peak levels during	**Frequency SCE and lymphocyte transformation rate (LTR) (%) (Mean+SEM), Change over 8 wks**

Reference and study design	Exposure	Results
students (11 males, 12 females, age not reported) exposed during 8-week course, 3-hr session, 3 times/ wk. **Outcome:** SCE in peripheral blood lymphocytes, assessed before the start of the course and at the end of 8-week period. Blinded analysis of slides, one observer with repeat by second; 30 M_2 lymphocytes per subject analyzed; Lymphocyte transformation rate (LTR)	class and in the dorms. Anatomy labs: Mean 3-hr TWA: 0.51 ± 0.299 mg/m^3, range: 0.07–1.28 mg/m^3 Dormitories: Mean TWA: 0.012 ± 0.003 mg/m^3, range: 0.011–0.016 mg/m^3 Duration: 8 wks	Before exposure / After exposure SCE: 6.383 ± 0.405 / 6.613 ± 0.786 LTR: 59.07 ± 6.35 / 56.92 ± 8.64 *p <0.05, paired t-test Levels in males and females were similar
He et al. (1998) China Prevalence study **Population:** 13 anatomy students exposed during a 12-week course compared to 10 students. Age and gender similar between groups, all nonsmokers (data not shown). **Outcome:** CA and SCE in peripheral lymphocytes, CA: modified fluorescence plus Giemsa stain, cells harvested 48 hr, scored 100 metaphases/ subject. SCE: cells harvested 72 hr, 50 metaphases/ subject. Blinding not described	Breathing zone air samples in location of exposed students. Concentration in breathing zone: Mean 2.92 mg/m^3 Duration: 12 weeks (10 hrs/wk)	**Frequency of SCE and chromosomal aberrations in peripheral lymphocytes** Referent / Exposed Mean SCE per cell: 5.26 ± 0.51 / 5.91 ± 0.71* Lymphocyte CA: 3.40 ± 1.57 / 5.92 ± 2.40* *p <0.05, analytic test not described
Suruda et al. (1993) USA Panel study **Population:** 29 students (with adequate samples) (24.1% female, mean age 23.6 yr, 17.2% smokers) exposed to formaldehyde for 9 wks during embalming course, with baseline samples taken. Mean duration of	Personal sampling for 121 of 144 embalmings; Exposure concentration: Mean: 1.72 mg/m^3 Range: (0.18–5.29) mg/m^3 Duration: 9 wks (0.173 yrs)	**Frequency of SCE before and after a 9-wk embalming course** Before exposure / After exposure SCE: 7.72 ± 1.26 / 7.14 ± 0.89* *p <0.01, difference in mean before and after exposure, matched Student's t-test

Reference and study design	Exposure	Results
embalming 125 min. Possible exposure prior to course. **Outcome:** SCE in peripheral lymphocytes, stain fluorescein plus Giemsa, 50 s division metaphases scored/ subject; blood samples collected in morning before 1st class and after 9 wks; analysis of slides blinded to exposure status		
Yager et al. (1986) USA Panel study **Population:** 8 anatomy students (1 male, 7 females, mean age 26 yr, all nonsmokers) exposed to formaldehyde during a 10 wk course (2 sessions/ wk). No occupational or lab formaldehyde exposure during previous year. **Outcome:** Mean SCEs per cell in peripheral lymphocytes; before and after 10 weeks, samples coded and randomized together for analysis	Ambient air and breathing zone monitoring. Breathing zone concentration: Mean:1.5 mg/m^3 Range: 0.9–2.4 mg/m^3 Exposure duration: 10 wks	**Mean SCE per cell before and after 10-wk course (mean ± SEM)** Before: Mean SCE per cell: 6.39 ± 0.11 After: Mean SCE per cell: 7.20 ± 0.33* *p = 0.02, paired t-test
Zeller et al. (2011) Germany Controlled human exposure study **Subjects:** 41 healthy volunteers exposed 4 hr/d for 5 d, all male, nonsmokers **Outcome:** SCE in peripheral lymphocytes: method according to Schmid and Speit (2007), scored 30 cells/ sample. Proliferation index (PI)	12 groups of 2 to 4 persons in a chamber, exposures randomly assigned. Formaldehyde concentrations: 0 (i.e., background level of 0.01 ppm), 0.3 ppm (0.37 mg/m^3)[a] with four peaks of 0.6 ppm (0.74 mg/m^3), 0.4 ppm (0.49 mg/m^3) with four peaks of 0.8 ppm (0.98 mg/m^3) and 0.5	**Frequency of SCE/ metaphase and PI in lymphocytes before and after 4-hour exposure (N = 40)** Lymphocytes: Before: SCE/ metaphase: 6.1 ± 0.898[a]; PI: 2.46 ± 0.114 Lymphocytes: After: SCE/ metaphase: 6.1 ± 0.938; PI: 2.47 ± 0.145 [a]p = 0.689

Reference and study design	Exposure	Results
calculated from 1st, 2nd, and 3rd mitoses in 100 metaphases. Analyzed using Wilcoxon Sign Rank test	ppm (0.67 mg/m^3) and 0.7 ppm (0.86 mg/m^3), peaks 15 min each, 4 15-min exercise sessions during exposure.	
Chromosomal Breaks or Aneuploidy		
Prevalence Studies		
Aglan and Mansour (2018) Egypt Prevalence study, June 2015 - September 2016 **Population:** 60 hair stylists who routinely conducted hair straightening compared to 60 stylists who did not conduct this treatment. Excluded subjects with chronic disease and /or regular medications, family history of cancer, recurrent abortions, smoking or pregnancy. Ages 20 – 36 years. **Outcome:** Blood collected at end of 8-hour shift. CB Micronucleus test in lymphocytes. Replicate cultures for each sample, incubated 72 hours. 2,000 binucleasted cells from coded slides (1,000 from each replicate culture), scored using criteria by Fenech et al. (2003). MN frequency % altered cells. MN in exfoliated buccal cells. Cheeks scraped with wooden spatula, fixed in 3:1 methanol/ acetic acid and dropped onto slides, stained with Feulgen/ Fast Green, examined at 400× according to Tolbert et al. (1991). Analyzed	Passive air sampling (Umex-100) at fixed position in breathing zone, 15-minute samples during hair straightening process; 15-minute TWA Group 1 (work duration < 5 years): 1.68 ± 0.27 ppm Group 2 (work duration > 5 years): 1.83 ± 0.16 ppm	**MN frequency (%) in PBL and buccal cells by duration of employment (< 5 and > 5 years)** PBL: Mean ± SD; EBC: Mean ± SD Referent (n=60): 0.22 ± 0.42*; 0.17 ± 0.38** < 5 years (n=31): 0.61 ± 0.50; 0.32 ± 0.48 > 5 years (n=29): 1.66 ± 0.48; 0.94 ± 0.58 $^*p < 0.01$, $^{**}p < 0.001$, Kruskal Wallis test Between group differences statistically significant in PBL and for EBC except between referent and < 5 year exposure group (least significant difference test)

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr><td>independently by 2 people, 1,500 cells scored per person using criteria by Sarto et al. (1987). % altered cells.</td><td></td><td></td></tr>
<tr><td>Costa et al. (2019)
Portugal
Prevalence study
extension of extension of Costa et al. (2015) adding outcomes
Population: 85 anatomy pathology workers from 9 hospital laboratories, exposed to formaldehyde for at least 1 yr, compared to 87 unexposed employees from administrative offices in same geographic area. Exclusions: cancer history, radiation therapy or chemotherapy, surgery with anesthesia or blood transfusion in last year. Exposed and referent similar for mean age 39 yrs, 77% females, 25% smokers. Outcome: Peripheral blood samples, coded, analyses blinded to exposure status. Exfoliated cells were collected for each cheek separately. Cytokinesis-blocked MN test, Costa et al. (2008); culture incubation 72 hr; stain 4% Giemsa; scored 1,000 binucleated cells/subject, criteria defined by Fenech (2007). Buccal MN cytome assay. 2,000 differentiated cells scored for frequency of MN, nuclear buds and</td><td>Exposure assessed via air sampling and deriving an 8-hr TWA for each subject.

Exposure concentration:
Mean: 0.38 ppm (0.47 mg/m^3)
Range: 0.28–1.39 ppm (0.34–1.72 mg/m^3)

Exposure duration
12.0 ± 8.2 years</td><td>MN frequency (%) in peripheral lymphocytes, exposed relative to referent group, Mean Ratio (MR)
<table><tr><th></th><th>Ratio</th><th>95% CI</th></tr><tr><td>Exposure</td><td>1.55**</td><td>1.2–1.99</td></tr></table>Poisson regression models adjusted for age, gender, smoking habits
**p <0.01
MN frequency in exfoliated buccal cells, Mean Ratio (MR)<table><tr><th></th><th>Exposed: Unexposed</th><th>MR</th><th>95% CI</th></tr><tr><td>MNB</td><td>63:69</td><td>4.08***</td><td>2.12 – 7.87</td></tr><tr><td>BNbud</td><td>63:69</td><td>2.88***</td><td>1.76 – 4.71</td></tr></table>Poisson regression models adjusted for age, gender, smoking habits; ***p < 0.001

Correlation between MNL and MNB: r = 0.359, p < 0.001

MN frequency in PBL and exfoliated buccal cells by level and duration in exposed, Mean Ratio (MR)<table><tr><th></th><th colspan="3">MNL</th><th colspan="3">BNbud</th></tr><tr><th></th><th>N</th><th>MR</th><th>95% CI</th><th>N</th><th>MR</th><th>95% CI</th></tr><tr><td>Level (ppm)</td><td></td><td></td><td></td><td></td><td></td><td></td></tr><tr><td>0.08-0.22</td><td>27</td><td>1.0</td><td></td><td>20</td><td>1.0</td><td></td></tr><tr><td>0.23-0.34</td><td>29</td><td>1.5**</td><td>1.12-2.00</td><td>16</td><td>1.42</td><td>0.64-3.14</td></tr><tr><td>0.35-1.39</td><td>28</td><td>1.37 *</td><td>1.04-1.81</td><td>17</td><td>1.96</td><td>0.91-4.24</td></tr><tr><td>Duration years</td><td></td><td></td><td></td><td></td><td></td><td></td></tr><tr><td>< 8</td><td>28</td><td>1.0</td><td></td><td>25</td><td>1.0</td><td></td></tr><tr><td>8-14</td><td>28</td><td>0.78</td><td>0.51-1.23</td><td>18</td><td>0.74</td><td>0.30-1.78</td></tr><tr><td>> 14</td><td>28</td><td>0.68</td><td>0.40-1.15</td><td>20</td><td>1.00</td><td>0.37-2.74</td></tr></table>Poisson regression models adjusted for age, gender, smoking habits
*p < 0.05; **p < 0.01.</td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr><td>nucleoplasmic bridges according to Tolbert et al. (1991) and Thomas et al. (2009).
T-Cell Receptor mutation assay in mononuclear leukocytes, flow cytometry, minimum of 2.5 × 105 lymphocyte-gated events were acquired, # events in mutation cell window (CD3-CD4+ cells) divided by total number of events for CD4+ cells</td><td></td><td></td></tr>
<tr><td>Wang et al. (2019)
Shanghai, China
Population: 100 male chemical production workers exposed to formaldehyde > 1 yr through 4 work processes (i.e., production examination, glue spraying, coating and workplace inspection). Unexposed group (n = 100 males) from the logistics workshop in same factory. Exposed and referent were comparable for mean age, smoking and alcohol consumption.
Outcome: CBMN according to Fenech (2000, 1993). Blinded analysis. Venous peripheral blood cultured for 44 hr, Cytochalasin-B added to cultures, cells harvested 28 hrs later, air dried slides stained with Giemsa, MN dectected at 400× with confirmation at 1,000×. 1,000 binucleated cells scored/ subject</td><td>Routine formaldehyde monitoring by factory
Range of geometric means (mg/m^3):
Exposed: 0.06–0.25
Unexposed: 0.01

Cumulative dose (mg/m^3-yr) determined for each worker (C × T). C = geometric mean of concentration for a year at a sampling site, T = yrs.
Exposed: 0.90 (0.60-1.78)
Referent: 0.06 (0.02-0.10)</td><td>MN frequency (% per 1,000, 95% CI) in PBLs
Exposed Referent
3.05 ± 1.47 1.71 ± 0.96
Poisson regression models adjusted for age, gender, smoking habits

Micronucleus frequency (per 1,000, frequency ratio (FR)) in PBL)
CED (mg/m^3-year) | N | Exposed | FR (95% CI)
0.01 – 0.06 | 45 | 1.36 ± 0.86 | 1
0.06 – 0.125 | 55 | 1.87 ± 0.92 | 1.38 (1.00-1.91)
0.125 – 0.9 | 46 | 2.50 ± 1.17 | 1.83 (1.34-2.52)
0.9 – 3.75 | 54 | 3.65 ± 1.40 | 2.67 (1.99-3.64)
Poisson regression models with adjustment for age, smoking status and alcohol use</td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr>
<td>Peteffi et al. (2015)
Brazil
Prevalence study
Population: 46 workers in furniture manufacturing facility (mean age 34.5 yr, 56.5% male, 1 smoker) and unexposed group (n = 45) recruited from employees and students of local university with no history of occupational exposure to potentially genotoxic agents or substances metabolized to formic acid. (mean age 35.4 yr, 33.3% male, 0 smokers)
Outcome: Oral buccal epithelial cell samples (scraped with endocervical brush), micronucleus test, DNA-specific Feulgen staining and counterstaining with Fast Green according to Tolbert et al. (1992); analyzed 2,000 cells/ person by 2 independent observers (1,000 ea).</td>
<td>Monitoring in 7 sections in facility; referent monitoring in 5 areas of university; breathing zone 8 hr samples collected on same day as biological samples. Urine samples collected at end of work day on 5th day of work; correlation of formaldehyde concentration in air with urinary formic acid concentration, r = 0.626, p <0.001

UV painting, lamination/press, packaging, edge lamination 0.03–0.04 ppm (0.037–0.05 mg/m^3)
Edge painting, machining and drilling center, board cutting 0.06–0.09 ppm (0.07–0.11 mg/m^3))

Referent mean (SD) 0.012 (0.008) ppm (0.015 (0.01) mg/m^3)
Formic acid median
Exposed 20.47 mg/L
Referent 4.57 mg/L
Exposure duration 5.76 yr</td>
<td>Comparisons of micronucleus frequency and other DNA damage in buccal cells, median (interquartile range)
<table>
<tr><th></th><th>Referent</th><th>Exposed</th><th>p-Value</th></tr>
<tr><td>Micronuclei</td><td>0</td><td>0</td><td>0.08</td></tr>
<tr><td>Nuclear buds</td><td>0 (0–0.50)</td><td>0.24 (0–0.63)</td><td>0.126</td></tr>
<tr><td>Binucleated cells</td><td>0.50 (0–1.38)</td><td>1.34 (0.64–2.38)</td><td>0.003</td></tr>
<tr><td>Karyorrhexis</td><td>1.0 (0.49–2.04)</td><td>1.31 (0.58–2.49)</td><td>0.372</td></tr>
</table>
Nonparametric tests used because data were not normally distributed. Exposed and referent compared using Mann-Whitney test.

No differences between men and women for measures of DNA damage in either exposed or referent.

No correlation between urinary formic acid and measures of DNA damage.</td>
</tr>
<tr>
<td>Souza and Devi (2014)
India
Prevalence study
Population: 30 male workers in anatomy departments (embalming) in several medical colleges (mean age 39.9 yr, 50% smokers); compared to 30 male clerical workers in same facilities (mean age</td>
<td>No measurements reported.

Duration exposure mean 10.66 yr, range 1–30 yr</td>
<td>MN frequency in Lymphocytes by Exposure Group (mean (SD))
<table>
<tr><th></th><th>Mean ± SD</th><th>95% CI</th></tr>
<tr><td>Exposed (N = 30)</td><td>9.5 ± 3.23</td><td>8.29–10.7</td></tr>
<tr><td>Comparison group (N = 30)</td><td>3.73 ± 1.43</td><td>3.19–4.26</td></tr>
<tr><td>Difference in means[a]</td><td>5.76</td><td>4.47–7.06[a]</td></tr>
</table>
[a]No difference = 0.</td>
</tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr><td>37.8 yr, 30% smokers).
Outcome: Total MN/ 1,000 cells in peripheral lymphocytes. Assays conducted blinded. Cytokinesis -blocked micronucleus assay Costa et al. (2008), 1,000 binucleated cells/ subject.</td><td></td><td>Association of MN frequency with exposure and smoking evaluated using two-way ANOVA. Smoking was not associated with MN frequency.

Pearson's correlation test showed a positive correlation (r = 0.5, P = 0.02) between the duration of exposure and the frequency of MN in lymphocytes.</td></tr>
<tr><td>Bouraoui et al. (2013)
Tunisia
Prevalence study
Population: 31 pathology workers (60% female, mean age 42, 9.6% smokers) compared to 31 unexposed administrative staff in same facility (60% female, mean age 43 yr, 12.9% smokers).
Outcome: MN peripheral lymphocytes: Cytokinesis-blocked MN assay in combination with FISH using all-chromosome centromeric probe Sari-Minodier et al. (2002); stain 5% Giemsa, 2,000 binucleated cells scored/ subject, Fenech (2000), blinding not described</td><td>Exposure assessed by job title and duration of employment. Atmospheric air sampling performed in area of potential exposure
Concentration:
Means of 3 samplings:
0.25 mg/m^3
2.21 mg/m^3
4.2 mg/m^3

Duration:
Mean 15.68 yrs (6.53 ± 0.7 hrs/day)</td><td>**MN frequency in peripheral lymphocytes (Mean ± SD)**
<table><tr><th></th><th>Referent</th><th>Exposed</th></tr><tr><td>MN (%/1,000 binucleated cells)</td><td>7.08 ± 4.62</td><td>25.35 ± 6.28*</td></tr><tr><td>FISH MN (%/ 2,000 cells)</td><td>6.12 ± 4.24</td><td>23.25 ± 5.92*</td></tr><tr><td>C + MN</td><td>4.03 ± 3.64</td><td>18.38 ± 5.94*</td></tr><tr><td>C − MN</td><td>2.09 ± 0.74</td><td>4.87 ± 3.22</td></tr><tr><td>C1+ MN</td><td>2.93 ± 2.74</td><td>15.35 ± 6.03*</td></tr><tr><td>Cx + MN</td><td>1.1 ± 1.16</td><td>3.03 ± 2.7*</td></tr></table>*p <0.05, Student's t-test

Duration of exposure was associated with all of the cytogenetic alterations.
Abbreviations: C +, C −, C1 + MN, Cx + MN</td></tr>
<tr><td>Costa et al. (2013)
Portugal
Prevalence study
Population: 35 pathology workers from 4 hospital laboratories, exposed to formaldehyde for at least 1 year (88.6% female, mean age 41.2 yr, 20% smokers), compared to 35 unexposed employees from same work area (80% female, mean age 39.8 yr, 20% smokers).
Outcome: MN in peripheral lymphocytes,</td><td>Exposure assessed via air sampling and deriving an 8-hr TWA for each subject.
Exposure conc.:
Mean 0.44 mg/m^3, range 0.28–0.85 mg/m^3

Exposure duration 12.5 ± 8.1 yrs, range 1–30 yr</td><td>Univariate analyses presented in Figure 1 of the paper. MN frequency was 2.5-fold higher in exposed group compared to referent group.

MN frequency (%) in peripheral lymphocytes, exposed relative to referent group
<table><tr><th></th><th>Ratio</th><th>95% CI</th></tr><tr><td>Exposure</td><td>2.1</td><td>1.025–3.174</td></tr></table>Multivariate analysis, adjusted for gender, smoking and age</td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr><td>samples collected between 10 & 11 am. Cytokinesis-blocked MN test Teixeira et al. (2004). 1,000 cells analyzed/ subject, MN per 1,000 binucleated cells, scored blindly by one reader, criteria Fenech (2007)

Related references: Costa et al. (2011); Costa et al. (2008)</td><td></td><td></td></tr>
<tr><td>Lin et al. (2013) China
Prevalence study
Population: 96 plywood workers exposed to formaldehyde (13.5% female, mean age 33 yr, 30.2% smokers) compared to referent group (N = 82) (4% female, mean age 31 yr, 40% smokers).
Outcome: MN assay in peripheral lymphocytes, analyzed 1,000 binucleated cells/ subject, scoring criteria Fenech (1993), Fenech et al. (2003), blinded analysis MN assessed by exposure group and # years worked.</td><td>Personal air monitoring and job assignment.

Average concentration:
High, N = 38 (making glue): 1.48 mg/m^3, range 0.914–2.044 mg/m^3
Low, N = 58 (sanding boards, pressing wood scraps with glue at high temp): 0.68 mg/m^3, range 0.455–0.792 mg/m^3
Referent group, N=82 (grinding wood scraps): 0.13 mg/m^3, range 0.019–0.252 mg/m^3
Exposure duration: 2.52 yrs</td><td>MN Frequency in peripheral lymphocytes by formaldehyde exposure level and work years

By Exposure levels
<table><tr><td></td><td>Referent</td><td>Low</td><td>HIgh</td></tr><tr><td>MN freq (%)</td><td>2.05 ± 1.72</td><td>2.02 ± 1.81</td><td>2.37 ± 1.79</td></tr></table>ANOVA p-value = 0.455; Poisson regression p-value = 0.288
Number of Work Years
<table><tr><td></td><td><1 (N= 57)</td><td>1–3 (N= 64)</td><td>>3 (N= 57)</td></tr><tr><td>MN freq (%)</td><td>1.02 ± 1.10</td><td>2.25 ± 1.56*</td><td>2.90 ± 1.96*</td></tr></table>ANOVA p-value < 0.001; Poisson regression p-value < 0.001
ANOVA and Poisson regression adjusting for age, gender, smoking status, alcohol, duration of employment</td></tr>
<tr><td>Costa et al. (2011)
Portugal
Prevalence study
Population: 48 pathology workers from 5 hospital laboratories, exposed for at least 1 year (28% female, mean age 40 yr, 21% smokers), compared to 50 unexposed</td><td>Exposure assessed via air sampling in breathing zone and deriving an 8-hr TWA for each subject.
Concentration:
Mean: 0.53 mg/m^3, range 0.05–1.94 mg/m^3</td><td>MN frequency (%) in peripheral lymphocytes
<table><tr><td></td><td>Referent</td><td>Exposed</td></tr><tr><td>MN</td><td>3.66 ± 0.51</td><td>6.19 ± 0.62*</td></tr></table>*p <0.05; Mann-Whitney U test and Kruskal-Wallis test</td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr><td>employees matched by age, gender, lifestyle, smoking habits and work area (25% female, mean age 37 yr, 14% smokers). Outcome: MN in peripheral blood lymphocytes, (Teixeira et al., 2004); stain 4% Giemsa; scored 1,000 binucleated cells/ subject, scored blind by one reader, criteria Fenech (2007)</td><td>Duration:
Mean: 13.6 yrs, range: 1−31 yr</td><td></td></tr>
<tr><td>Ladeira et al. (2011)
Portugal
Prevalence study
Population: 56 hospital workers in histopathology labs (66% female, mean age 39.5 yr, 19.6% smokers) compared to 85 administrative staff (64% female, mean age 32.4 yr, 29.4% smokers).
Outcome: MN in peripheral lymphocytes and buccal cells. Samples coded and analyzed blinded. Lymphocytes, cytokinesis-block micronucleus cytome assay, stain May-Grunwald-Giemsa, 1,000 binucleated cells scored/ subject by 2 readers; buccal mucosa cells, stain Feulgen, 2,000 cells scored/ subject, 2 readers

Related references: Speit et al. (2012); Viegas et al. (2010)</td><td>Personal air sampling, 6−8 hrs, estimated 8-hr TWA
Exposure conc.:
Mean TWA 8 hr 0.2 ± 0.14 mg/m^3
Mean ceiling value: 1.4 ± 0.91 mg/m^3, range 0.22−3.6 mg/m^3

Exposure duration: 14.5 (1−33) yrs</td><td>MN frequency (Mean ± SD) by cell type
<table><tr><th></th><th>Lymphoctyes</th><th>Buccal cells</th></tr><tr><td>Referent</td><td>0.81 ± 0.172</td><td>0.16 ± 0.058</td></tr><tr><td>Exposed</td><td>3.96 ± 0.525*</td><td>0.96 ± 0.277*</td></tr><tr><td>OR[a]</td><td>9.67</td><td>3.99</td></tr><tr><td>95% CI</td><td>3.81−24.52</td><td>1.38−11.58</td></tr></table>*p≤0.002, Mann-Whitney test
[a]Odds ratio for risk of presence of MN; binary logistic regression

MN frequency (Mean ± SD) by years of exposure
<table><tr><th>Years</th><th>N</th><th>Lymphocytes</th><th>Buccal cells</th></tr><tr><td><5</td><td>8</td><td>2.75 ± 0.940</td><td>0.63 ± 0.625</td></tr><tr><td>6−10</td><td>19</td><td>3.05 ± 0.775</td><td>0.63 ± 0.326</td></tr><tr><td>11−20</td><td>12</td><td>5.50 ± 1.317</td><td>0.83 ± 0.458</td></tr><tr><td>>21</td><td>15</td><td>5.00 ± 1.151</td><td>1.20 ± 0.8</td></tr></table>Evaluated potential confounding by age, gender, smoking and alcohol, no major evidence of confounding noted</td></tr>
<tr><td>Jiang et al. (2010)
China
Prevalence</td><td>Exposure assessed by job title and personal air monitoring.</td><td>Lymphocyte MN frequency by duration and formaldehyde concentration
<table><tr><th>Duration (yrs)</th><th>MN[a]</th><th>Conc. (mg/m^3)</th><th>MN[b]</th></tr></table></td></tr>
</table>

Reference and study design	Exposure	Results
Population: 151 male workers from 2 plywood plants (mean age 27.4 yr, 52.3% smokers) compared to 112 unexposed workers at a machine manufacturer in same town (mean age 28.7 yr, 42.9% smokers). **Outcome:** Cytokinesis-block micronucleus (CB-MN), Fenech (1993), scoring criteria Fenech et al. (2003), 1,000 binucleated lymphocytes/ subject, blinded analysis	Exposure concentration ppm converted to mg/m^3 by EPA. Exposed: 1.08 mg/m^3, range 0.1–7.75 mg/m^3 Referent: <0.01 mg/m^3 (LOD) Duration: Mean 2.51 yrs Range: (0.5–25) yrs	0.6–1 | 4.33 ± 2.81 | 0.0123[c] | 2.67 ± 1.32 1–3 | 5.84 ± 3.63 | 0.1353 | 4.03 ± 2.40 3–25 | 5.84 ± 3.24* | 0.3444 | 5.74 ± 3.13* | | 0.4797 | 6.76 ± 3.81* | | 3.1488 | 8.25 + 3.53* [a]ANOVA, Dunnett-Hsu test, p =0.04, adjusted for age, formaldehyde concentration, current smoking status, alcohol [b]ANOVA, p <0.05; Trend p <0.001 [c]Referent group
Viegas et al. (2010) Portugal Prevalence study **Population:** 30 formaldehyde factory workers and 50 pathology/anatomy lab workers exposed for >1 year (40% female, mean age 35.7 yr, 31.3% smokers), compared to 85 unexposed individuals (63.5% female, mean age 33.9 yr, 30.6% smokers) **Outcome:** MN assay, buccal mucosa cells and peripheral lymphocytes. Blinded coding and analysis, Buccal cells, Feulgen stain, 2,000 cells scored/ subject by 4 observers, scoring criteria Tolbert et al. (1992), peripheral lymphocytes, stain May-Grunwald-Giemsa, 1,000 binucleated cells scored/ subject Also discussed in Viegas et al. (2013)	Personal air sampling, (N=2 in factory, N=29 in labs) 6–8 hrs, estimated 8-hr TWA Exposure duration: Factory workers: 6.2 (1–27) yr Lab workers: 14.5 (1–33) yr 8-hr TWA Concentration in: Factory: 0.26 mg/m^3, range 0.25–0.27 mg/m^3 Lab: 0.34 mg/m^3, range 0.06–0.63 mg/m^3 Ceiling Concentrations Factory: 0.64 mg/m^3, range 0.004–1.28 mg/m^3 Lab: 3.1 mg/m^3, range 0.03–6.18 mg/m^3	**MN Frequency by cell type (mean ± SD)** Referent | Factory | Laboratory Peripheral lymphocytes | 1.17 ± 1.95 | 1.76 ± 2.07 | 3.7 ± 3.86* Buccal cells | 0.13 ± 0.48 | 1.27 ± 1.55* | 0.64 ± 1.74* *p <0.01, Spearman's correlation test Years of exposure correlated with MN in peripheral lymphocytes (r = 0.401, p <0.01), and MN in buccal cells (r = 0.209, p = 0.008); Spearman's test No correlation between MN frequency and smoking or gender, small magnitude of correlation with age (r = +0.194; p <0.05 for blood lymphocytes, r = -0.168; p <0.05 for buccal cells).
Costa et al. (2008) *Portugal*	Air sampling in breathing zone,	**MN frequency in peripheral lymphocytes** Referent | Exposed

Reference and study design	Exposure	Results
Prevalence study **Population:** 30 pathology lab workers (4 hospitals), (70% female, mean age 38 yr, 27% smokers) compared to 30 administrative employees matched by age, gender, lifestyle, smoking habits, and work area (63.3% female, mean age 37 yrs, 23% smokers). **Outcome:** MN in peripheral lymphocytes (Teixeira et al., 2004), stain 4% Giemsa; scored 1,000 binucleated cells/ subject, scored blind by one reader, criteria Caria et al. (1995)	derived an 8-hr TWA for each subject Concentration: Mean: 0.54 mg/m^3, range: 0.05−1.94 mg/m^3 Duration: 11 yrs Range: (0.5−27) yrs	Lymphocyte MN: 3.27 ± 0.69 vs 5.47 ± 0.76* *P*=0.003, Mann-Whitney U-test and Kruskal-Wallis test. Authors reported positive correlation between formaldehyde exposure levels and MN frequency (*r*=0.384, *p*=0.001)
Pala et al. (2008) Italy Prevalence study **Population:** 36 lab workers (66.7% female, mean age 40.1 yr, 16.7% smokers) **Outcome:** Peripheral lymphocytes (blood sampled at end of 8-hour shift), analysis blind to exposure. MN using modified cytokinesis-blocked method, Fenech and Morley (1986); stain 3% Giemsa, 2,000 cells/ subject	Personal air monitoring (8-hr sample); Exposure categories: High: ≥ 0.026 mg/m^3, Low: < 0.026 mg/m^3 Mean concentration: Low (*n* = 25): 0.015 mg/m^3 (range 0.005−0.0254) High (*n* = 9): 0.056 mg/m^3 (range 0.026−0.269) Duration of exposure: NR	**Micronuclei Frequency by Exposure Level (mean ± SD)** MN: <0.026 mg/m^3: 0.26 ± 0.24; ≥0.026 mg/m^3: 0.31 ± 0.17 Means ratio (95% CI) 1.43 (0.26−7.81), Poisson regression adjusted for gender, age, smoking and other exposures
Orsiere et al. (2006) *France* Prevalence **Population:** 59 hospital pathology workers from 5 labs (81% female, mean age 44.7 yr, 20% smokers) compared to 37 unexposed workers (76% female, mean age 44 yr, 24% smokers).	Personal sampling; Short-term: 15 min, Long-term 8 hrs during typical work-day. Concentration[1]: Mean 15-min: 2.46 mg/m^3, range <0.12−25. 1 mg/m^3	**Binucleated micronucleated cell rate (BMCR) in peripheral lymphocytes (mean ± SD)** % BMCR: Unexposed (n=37) 11.1 ± 6.0; Exposed (*n*=59) 16.9 ± 9.3* *Number BMCR per 1,000 binucleated cells, *p*<0.05, Mann-Whitney U-test. Linear regression of BMCR, increase of 0.263 per 1,000 binucleated cells in exposed, *p* =0.003, adjusting for gender, age, smoking and alcohol.

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr><td>Outcome: MN in peripheral lymphocytes. Subgroups selected randomly from initial groups. Assays conducted blinded. Cytokinesis-blocked micronucleus assay Sari-Minodier et al. (2002); stain 5% Giemsa, scoring criteria Fenech (2000), 1,000 binucleated cells/ subject; FISH with a pan-centromeric DNA probe, same operator scored exposed and referent blinded

Related reference: Iarmarcovai et al. (2006).</td><td>Mean 8-hour 0.123 (range <0.123−0.86 mg/m^3

Duration exposure 13.2 yrs, range 0.5−34 yrs</td><td>FISH Analysis of MN in peripheral lymphocytes by exposure (mean ± SD)
<table><tr><th>FISH Results[1]</th><th>Unexposed (n= 18)</th><th>Exposed (n = 18)</th><th>p-Value</th></tr><tr><td>% BMCR</td><td>11.9 ± 5.6</td><td>19.1 ±10.1</td><td>0.021</td></tr><tr><td>% MN</td><td>14.4 ± 8.1</td><td>21.0 ± 12.6</td><td>0.084</td></tr><tr><td>C + MN (%)</td><td>10.3 ± 7.1</td><td>17.3 ± 11.5</td><td>0.059</td></tr><tr><td>C − MN (%)</td><td>4.1 ± 2.7</td><td>3.7 ± 4.2</td><td>0.338</td></tr><tr><td>C1 + MN (%)</td><td>3.1 ± 2.4</td><td>11.0 ± 6.2</td><td>p<0.001</td></tr><tr><td>Cx + MN (%)</td><td>7.8 ± 5.5</td><td>6.3 ± 6.3</td><td>0.163</td></tr></table>[1]Results expressed as frequency per 1,000 binucleated cells, mean ± SD; analyzed using Mann-Whitney U-test
Linear regression of C1 + MN, increase of 0.586 MN containing one centromere per 1,000 binucleated cells in exposed, <0.001, adjusting for gender, age, smoking and alcohol</td></tr>
<tr><td>Ye et al. (2005) China
Prevalence study
Population: 18 workers at a formaldehyde plant at least 1 yr (38.9% female, mean age 29 yr, and 16 workers exposed to indoor air formaldehyde via building materials (75% female, mean age 22 yr) compared to 23 students with no known source of formaldehyde exposure (dormitories) (48% female, mean age 19 yr); all nonsmokers
Outcome: MN in nasal cells, stain Wright's, scoring criteria Fenech et al. (2003), per 3,000 cells, blinding not stated.</td><td>Formaldehyde sampling: TWA
Concentration
Controls
0.011 ± 0.0025 mg/m^3
Max. 0.015 mg/m^3
Wait staff
0.107 ± 0.067 mg/m^3
Max. 0.30 mg/m^3
Workers
0.985 ± 0.286 mg/m^3
Max. 1.694 mg/m^3
Exposure duration:
Workers 8.5 (1−15) yrs
Waiters 12 wks</td><td>MN frequency in nasal cells
<table><tr><th></th><th>Referent</th><th>Wait Staff</th><th>HCHO Workers</th></tr><tr><td>MN</td><td>1.25 ± 0.65</td><td>1.75 ± 1.00</td><td>2.70 ± 1.50*</td></tr></table>P <0.05, one-way ANOVA, values estimated from figure</td></tr>
<tr><td>Burgaz et al. (2002)
Turkey
Prevalence study</td><td>Concentration:
Range:2.46−4.92 mg/m^3</td><td>MN frequency (%) in buccal mucosal cells (mean ± SD)
<table><tr><th></th><th>Referent</th><th>Exposed</th></tr><tr><td>MNF Frequency</td><td>0.33 ± 0.30</td><td>0.71 ± 0.56*</td></tr></table></td></tr>
</table>

Reference and study design	Exposure	Results
Population: 28 pathology workers (46.4% female, mean age 29.7 yr, 43% smokers) and 18 unexposed male employees (mean age 31.1 yr, 25% smokers), may overlap with study population from Burgaz et al. (2001) **Outcome:** MN frequency in buccal mucosal cells, stain Feulgen's reaction plus Fast Green, MN, 3,000 cells/ subject counted, coded slides, scoring criteria Tolbert et al. (1992); Sarto et al. (1987)	Duration: 4.7 ± 3.33 (1–13) yrs	*p <0.05, multifactorial ANOVA adjusting for age, smoking, and gender MN frequency was not associated with duration of exposure
Burgaz et al. (2001) Turkey Prevalence study **Population:** 23 pathology workers (12 male, 11 female) occupationally exposed 5 d, 8 hrs/ wk, mean age 30.6 yr, 39% smokers compared to 25 male university and hospital staff, mean age 35.4 yr, 76% smokers **Outcome:** MN frequency in nasal cells. Previously coded slides, stain Feulgen's reaction plus Fast Green, MN, 3,000 cells/ subject counted, scoring criteria Tolbert et al. (1992); Sarto et al. (1987)	Exposure based on occupation and duration of employment and quantified via stationary air monitors Exposure conc.: 2.46–4.92 mg/m^3 (converted from ppm by EPA) Exposure duration: Mean: 5.06 ± 3.47 Yrs Range: (1–13) yrs	**MN frequency (%) in nasal epithelial cells (mean ± SD)** Referent: MN frequency 0.61 ± 0.27; Exposed: 1.01 ± 0.62* *p <0.05, nonparametric test MN frequency was not associated with duration of exposure. MN frequency higher in male exposed, similar between smokers and nonsmokers in referent.
He et al. (1998) China Prevalence study **Population:** 13 anatomy students exposed during a 12-wk course (10 hr/ wk) compared to 10 students	Breathing zone air samples during dissection. Measurements limited to location of exposed students.	**MN frequency (%) in peripheral blood lymphocytes (mean ± SD)** Referent: Lymphocyte MN 3.15 ± 1.46; Exposed: 6.38 ± 2.50* *p <0.01, analytic test not described

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr><td>from same school. Age and gender similar between groups, all non-smokers.
Outcome: MN assay, Fenech and Morley (1985), scored 1,000 cells per individual, blinding not described</td><td>Concentration in breathing zone: Mean 3.17 mg/m^3
Duration:
12 wks (10 hrs/wk)</td><td></td></tr>
<tr><td>Kitaeva et al. (1996)
Russia
Prevalence study
Population: anatomy instructors (8 female, 5 male), mean age 41 yr) compared to 6 female unexposed (mean age 28.5 yr); students (6 female, 6 male)
Outcome: MN in buccal cells, 1994–95. MN in mucosal cells compared between exposed and referent instructors, and before and after a 40-min exposure for students at 24 and 48 hrs. Blinding not described, stain Feulgen and light green, analyzed 2,000 cell/ subject</td><td>No quantitative exposure assessment. Duration of employment among instructors, females 23.6 yrs; males 25.6 yrs
17 yrs
40-min exposures</td><td>**MN frequency (%) in buccal mucosa cells**
<table><tr><th></th><th>Referent</th><th>Exposed</th><th></th></tr><tr><td>Female instructors</td><td>0.64 (N=6)</td><td>2.94* (N=8)</td><td></td></tr><tr><td></td><td>Before</td><td>24 Hr Post</td><td>48 Hr Post</td></tr><tr><td>Female students</td><td>0.58</td><td>2.50**</td><td>2.64**</td></tr><tr><td>Male students</td><td>0.77</td><td>2.02*</td><td>1.86</td></tr></table>*p <0.05, **p <0.01, Student's t-test</td></tr>
<tr><td>Ballarin et al. (1992)
Italy
Prevalence study
Population: 15 plywood factory workers (46.7% female, mean age 31 yrs,) compared to 15 university or hospital clerks matched for age and sex (mean age 31 yr). All nonsmokers.
Outcome: MN in nasal mucosal cells, stain feulgen's plus Fast Green, analysis blinded by one reader, 6,000 cells/</td><td>Personal sampling; 8-hr TWA (NIOSH, 1977)
Warehouse (N=3) 0.39 ± 0.20 mg/m^3, range 0.21–0.6 mg/m^3
Shearing-press (N=8) 0.1 ± 0.02 mg/m^3, range 0.08–0.14 mg/m^3
Sawmill (N=1), 0.09 mg/m^3
Inspirable wood dust: 0.11–0.69 mg/m^3, 0.73 in sawmill</td><td>**Mean frequency micronuclei per 1000 cells in nasal mucosal cells by exposure group**
<table><tr><th></th><th>Referent</th><th>Exposed</th></tr><tr><td>MN (%) (SD)</td><td>0.25 (0.22)</td><td>0.9 (0.47)*</td></tr></table>*p <0.01, Mann-Whitney U test</td></tr>
</table>

Reference and study design	Exposure	Results
subject, scoring criteria Sarto et al. (1987).	Employment duration 6.8 yrs	
Short-term Studies		
Lin et al. (2013) China Cross-shift change **Population:** 62 plywood workers (17.7% female, mean age 34 yr, 17.7% smokers) **Outcome:** Peripheral lymphocytes, cytokinesis-block micronucleus assay, Fenech (1993), analyzed 1,000 binucleated cells/ subject, scoring criteria Fenech (1993), Fenech et al. (2003); blinded analysis	Air sampling and job function. Mean exposure: 0.27 ± 0.20 mg/m^3, range: 0.012–0.67 mg/m^3 Mean exposure duration 2.53 ± 2 yr	**Frequency micronuclei in binucleated cells in peripheral lymphocytes** MN (%): Before exposure 2.29 ± 1.21; After exposure 2.29 ± 1.65 p = 0.754, paired Wilcoxon test Regression coefficients for formaldehyde level, before shift 0.73 (−0.46, 1.92); after shift −0.01 (−1.38, 1.35) Poisson regression adjusted for age, gender, smoking, and alcohol
Ying et al. (1997) China Panel study **Population:** 25 non-smoking anatomy students (13 males, 12 females, mean age 18.8 yr, Han nationality) exposed during 8-wk course, 3-hr session, 3 times/ wk. **Outcome:** MN Nasal and Buccal cells, assessed before the start of the course and at the end of 8-wk period. Blinded analysis, one observer; Wright's stain, scored 4,000 cells/ subject; MN blood lymphocytes, stain 4% Giemsa, scored mean of 2870–3167 cells/ subject; MN scoring criteria Sarto et al. (1987)	Air sampling, estimated TWA and peak levels during class and in the dorms. Anatomy labs: Mean TWA: 0.51 ± 0.299 mg/m^3, range: 0.07–1.28 mg/m^3 Dormitories: Mean TWA: 0.012 ± 0.003 mg/m^3, range: 0.011–0.016 mg/m^3 Duration: 8 eks	**Micronucleated Cell Frequency (Mean+SEM), Change over 8 weeks** Oral Mucosa: Before exposure 0.57 ± 0.32; After exposure 0.86 ± 0.56* Nasal Mucosa: Before exposure 1.20 ± 0.67; After exposure 3.84 ± 1.48* Lymphocytes: Before exposure 0.91 ± 0.39; After exposure 1.11 ± 1.54 *p <0.01, paired t-test
Titenko-Holland et al. (1996) USA Panel study	See Suruda et al. (1993)	**Micronuclei before and after embalming class (per 1,000 cells) by cell type** Preexposure Postexposure

Reference and study design	Exposure	Results
Population: same subjects as in Suruda et al. (1993); 35 mortuary students intermittently exposed for 90 d (28 students (with adequate samples, 22 males, 6 females)), age 20–33 yrs. **Outcome:** MN analysis on buccal and nasal cells using FISH; blinded analysis Related study: Suruda et al. (1993), same subjects	Subjects with complete MN data from buccal mucosa cells (n=19): Lagged (7–10 d before the last sampling): 1.2 ± 2.1 ppm-hrs; 90-d cumulative (90 d): 14.8 ± 7.2 ppm-hrs; Subjects with complete MN data from nasal cells (n=13): Lagged (7–10 d): 1.9 ± 2.5 ppm-hrs; 90-day cumulative (90 days): 16.5 ± 5.8 ppm-hrs	*Buccal Cells (N = 19)* MN Total 0.6 ± 0.5 2.0 ± 2.0* MN^+ 0.4 ± 0.4 1.1 ± 1.3 MN^- 0.1 ± 0.2 0.9 ± 1.1* *Nasal Cells (N = 13)* MN Total 2.0 ± 1.3 2.5 ± 1.3 MN^+ 1.2 ± 1.3 1.0 ± 0.8 MN^- 0.5 ± 0.5 1.0 ± 0.6* **p* <0.05, Wilcoxon sign-rank test, two-tailed Association with 90-d cumulative exposure for change in total MN frequency in buccal cells, *r* =0.44, *p* =0.06; no association with 7–10 d lagged exposure, Spearman rank order correlation
Suruda et al. (1993) USA Panel study **Population:** 29 students (with adequate samples) (24.1% female, mean age 23.6 yr, 17.2% smokers) exposed to formaldehyde for 9 weeks during embalming course, with baseline samples taken. Mean duration of embalming 125 min. Possible exposure prior to course. **Outcome:** MN assay, nasal, buccal and micronucleated peripheral blood lymphocytes. Analysis blinded to exposure status; MN assay buccal and nasal cells, Stich et al. (1982), stain Feulgen/ Fast Green, 1,500 cell/ subject; MN lymphocytes Fenech and Morley (1985),	Personal sampling for 121 of 144 embalmings; cumulative exposure estimated using sampling data and time-activity data; Continuous area samples over embalming tables for short-term peaks; Concentration[1]: Mean: 1.72 mg/m^3, range 0.18–5.29 mg/m^3 Duration: 9 weeks Average cumulative exposure 18.2 mg/m^3–hr, range 5.3–41.3 mg/m^3-hr 8-hr TWA Mean 0.41 mg/m^3, range 0.123 – 1.2 mg/m^3 Measurements of glutaraldehyde, phenol, & methanol all < LOD, isopropyl alcohol < LOD or very low.	**Micronuclei before and after embalming class (per 1,000 cells)** Cell type	Before exposure	After 9 weeks Buccal 0.046 ± 0.17 0.60 ± 1.27* Nasal 0.41 ± 0.52 0.50 ± 0.67 Micronucleated lymphocytes 4.95 ± 1.72 6.36 ± 2.03* **p* <0.05, Wilcoxon sign-rank test Buccal MN in males associated with cumulative exposure, Spearman coefficient, not nasal MN or micronucleated lymphocytes

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr><td>stain Feulgen 2,000 cells/ subject</td><td></td><td></td></tr>
<tr><td>Zeller et al. (2011)
Germany
Controlled human exposure study
Subjects: 41 healthy volunteers exposed 4 hr/ day for 5 days, all male, nonsmokers
Outcome: MN in peripheral blood lymphocytes and nasal mucosa cells assessed before and after exposure. Lymphocytes: CBMN test, scored 1,000 binucleated cells/ subject on coded slides. Nuclear division index (NDI) = # cells with 1 − 4 micronuclei/ Total cells scored. Nasal cells: scored 2,000 cells/ subject on coded slides. Difference in means analyzed using Cochran Mantel Haentzel test and ANOVA.</td><td>12 groups of 2 to 4 persons in a chamber, exposures randomly assigned. Formaldehyde concentrations: 0 (i.e., background level of 0.01 ppm), 0.3 ppm (0.37 mg/m^3)[a] with four peaks of 0.6 ppm (0.74 mg/m^3), 0.4 ppm (0.49 mg/m^3) with four peaks of 0.8 ppm (0.98 mg/m^3) and 0.5 ppm (0.67 mg/m^3) and 0.7 ppm (0.86 mg/m^3), peaks 15 min each, 4 15-min exercise sessions during exposure.</td><td>Frequency of micronuclei and NDI in lymphocytes and nasal mucosa before and after 4-hour exposure over 5 d (N = 40)
<table><tr><th></th><th>Cells with micronuclei/ 1,000</th><th>Nuclear Division Index</th></tr><tr><td>Lymphocytes</td><td></td><td></td></tr><tr><td>Before</td><td>6.5 ± 3.226</td><td>2.0 ± 0.232</td></tr><tr><td>After</td><td>5.7 ± 3.339[a]</td><td>2.0 ± 0.176</td></tr><tr><td>Nasal mucosa[b]</td><td></td><td></td></tr><tr><td>Before</td><td>0.21 ± 0.35</td><td></td></tr><tr><td>After</td><td>0.27 ± 0.42</td><td></td></tr><tr><td>1-week after</td><td>0.24 ± 0.43</td><td></td></tr><tr><td>2-weeks after</td><td>0.24 ± 0.45</td><td></td></tr><tr><td>3-weeks after</td><td>0.17 ± 0.41</td><td></td></tr></table>[a] $p = 0.11$
[b]Several slides could not be analyzed, hence only 1,000 cells scored for several individuals (9−13 subjects per sampling time).</td></tr>
<tr><td>Speit et al. (2007a)
Germany
Controlled human exposure study
Subjects: 21 healthy volunteers exposed to formaldehyde for 4 hrs/d for 10 d, 11 males, nonsmokers, aged 19−36 years.
Outcome: MN in buccal mucosal cells assessed prior to controlled exposure and then during postexposure period. Blinded analysis at end of study by one person, stain DAPI/ propidium iodide, Analyzed 2,000 cells/ subject</td><td>Source: para-formaldehyde. Exposure duration: 10 consecutive d, 5 groups of 3−6 persons in chamber, 4-hour exposures, some exposures masked with ethyl acetate (EA), 3 15-min exercise sessions during exposure. Cumulative exposure 16.6 mg/m^3 − hrs; Target concentrations: 0, 0.15, 0.3, 0.5, 0 + EA, 0.3 + EA, 0.5 + EA, 0.3 + 4 x 0.6, 0.5 + 4 x 1.0, and 0.4 + 4 x 1.0 + EA</td><td>MN Frequency (per 1,000 cells) in Buccal Mucosa, mean ± SD
<table><tr><th></th><th>Immediately before exposure</th><th>End of 10-d exposure</th></tr><tr><td>Mean MN</td><td>0.86 ± 0.84</td><td>1.33 ± 1.45</td></tr></table>$p = 0.052$, Wilcoxon signed rank test</td></tr>
</table>

Reference and study design	Exposure	Results
DNA Damage		
Prevalence Studies		
Zendehdel et al. (2017) Iran Prevalence study **Population:** Workers in 3 melamine dinnerware manufacturing workshops (n=49) and referents matched by age and sex (n=34) who worked in food industries, # smokers higher in referent (26% versus 16%), >90% male. Recruitment and participation were not described. **Outcome:** Peripheral blood cells, Comet assay, alkaline conditions, according to Tice et al. (2000) blinding not described; minimum of 50 randomly selected cells per sample; tail moment and Olive moment	Personal air sampling, NIOSH method 3500, whole shift for each worker. Median TWA in 3 workshops, 0.086 mg/m^3; range, 0.02–0.22 mg/m^3; authors state that 2/3 of sample were exposed to < 0.1 mg/m^3 Work duration: Exposed 2.5 (1–22) yrs Referent 2.0 (1–25) yrs	Comparison of DNA damage (comet assay) between exposed and referent Olive moment Median (min-max) / Tail moment Median (min-max) Exposed (N = 49): 13 (7.4-36.7) / 22.2 (12.3-65) Referent (N = 34): 8.4 (6.4-31.7) / 14.8 (6.4-57.7) *p* value = 0.001; Mann-Whitney test
Costa et al. (2015) Portugal Prevalence study **Population:** 83 anatomy pathology workers from 9 hospital laboratories, exposed to formaldehyde for at least 1 yr, compared to 87 unexposed employees from administrative offices in same geographic area. Exclusions: cancer history, radiation therapy or chemotherapy, surgery with anesthesia or blood transfusion in last year. Exposed and referent similar for mean age 39 yrs, 77% females, 25%	Exposure assessed via air sampling and deriving an 8-hr TWA for each subject. Exposure concentration: Mean: 0.38 ppm (0.47 mg/m^3) Range: 0.28–0.85 ppm (0.34-1.05 mg/m^3) Exposure duration 12.0 ± 8.2 yrs	Comparison of % DNA in tail (comet assay) between exposed and referent Exposed (N = 83): Mean 11.67[a] / SD 0.72 / Mean Ratio (95% CI) 1.5 (1.14–1.96)[b] Referent (N = 87): 7.5 / 0.47 / 1.0 [a]Student's *t*-test, $p<0.001$. [b]model adjusted for age, gender, smoking habit, and fruit consumption (# pieces consumed/d).

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr><td>smokers. Outcome: Peripheral blood samples, coded, analyses blinded to exposure status.
Comet assay: alkaline conditions according to Singh et al. (1988); Scored blind 100 cells/ donor from two gels; % DNA in comet tail.
Exposed compared to unexposed using Student's t-test for ln % tDNA; linear regression of ln %tDNA</td><td></td><td></td></tr>
<tr><td>Peteffi et al. (2015)
Brazil
Prevalence study
Population: 46 workers in furniture manaufacturing facility (mean age 34.5 yr, 56.5% male, 1 smoker) and unexposed group (n = 45) recruited from employees and students of local university with no history of occupational exposure to potentially genotoxic agents or substances metabolized to formic acid. (mean age 35.4 yr, 33.3% male, 0 smokers)
Outcome: Peripheral blood processed within 4 hr. Comet assay, alkaline conditions according to Tice et al. (2000); silver nitrate staining according to Nadin et al. (2001); 100 cells/ person read by two independent observers (50 cells each), classified by visual scoring according to Anderson et al. (1994); 5 categories based on tail migration (0–IV) and frequency of damaged</td><td>Monitoring in 7 sections in facility; referent monitoring in 5 areas of university; breathing zone 8 hr samples collected on same day as biological samples. Urine samples collected at end of work day on 5th day of work; correlation of formaldehyde concentration in air with urinary formic acid concentration, r = 0.626, p<0.001

UV painting, lamination/press, packaging, edge lamination 0.03–0.04 ppm (0.037–0.05 mg/m^3)
Edge painting, machining and drilling center, board cutting 0.06–0.09 ppm (0.07–0.11 mg/m^3))

Referent mean (SD) 0.012 (0.008) ppm (0.015 (0.01) mg/m^3)
Formic acid median Exposed 20.47 mg/L</td><td>Comparisons of DNA damage (comet assay) in peripheral blood cells, median (interquartile range)
<table><tr><th></th><th>Referent</th><th>Exposed</th><th>p-Value</th></tr><tr><td>Damage index</td><td>2.0 (0–4.0)</td><td>6.5 (1.0–12.5)</td><td>0.007</td></tr><tr><td>Damage frequency (%)</td><td>2.0 (0–4.0)</td><td>6.0 (1.0–12.5)</td><td>0.003</td></tr></table>
No differences between men and women for measures of DNA damage in either exposed or referent.

No correlation between urinary formic acid and measures of DNA damage.</td></tr>
</table>

Reference and study design	Exposure	Results
cells (sum of I–IV), damage index (Pitarque et al., 1999) Nonparametric tests used because data were not normally distributed. Exposed and referent compared using Mann-Whitney test	Referent 4.57 mg/L Correlation formaldehyde concentration and formic acid r = −0.626, p <0.001 Exposure duration 5.76 yr	
(Aydın et al., 2013) Turkey Prevalence study **Population:** 46 male workers from 2 MDF plants (mean age 33.4 yr, 39.1% smokers) compared to 46 non-exposed male workers in same area (mean age 38.4 yr, 50% smokers) (administrative government offices and maintenance services). Half of workers used personal protective equipment. **Outcome:** DNA damage, Comet assay, tail intensity, tail moment, and tail migration, alkaline conditions, 100 cells/ subject	24 area samples in workplaces; personal samples in breathing zone over 8 hrs. Mean: 0.25 ± 0.07 mg/m^3 Range (0.12–0.41) Duration: Mean: 7.3 yrs Range (0.33–30)	**Comparison of Comet assay results in peripheral blood lymphocytes by exposure** Unexposed / Exposed Tail intensity: 5.28 ± 0.22 / 4.25 ± 0.29* Tail moment: 0.816 ± 0.002 / 0.624 ± 0.003* Tail migration: 2.16 ± 0.007 / 1.68 ± 0.005* *ANOVA, P <0.05. Comparisons by smoking strata indicate similar pattern.
Lin et al. (2013) China Prevalence study **Population:** 96 plywood workers exposed to formaldehyde (13.5% female, mean age 33 yr, 30.2% smokers) compared to referent group (N=82) (4% female, mean age 31 yr, 40% smokers). **Outcome:** Blood lymphocytes: DNA damage, Comet assay, olive tail moment, alkaline conditions (pH = 13), 50	Exposure assessed by air monitoring and job assignment. Average concentration: High Exposure, N=38 (making glue): 1.48 mg/m^3 (0.914–2.044) Low exposure, N=58 (sanding boards, pressing wood scraps with glue at high temp): 0.68 mg/m^3 (0.455–0.792) Referent group, N=82 (providing & grinding	**Comparison of Comet assay results in peripheral blood lymphocytes by exposure and duration of employment.** **By Exposure** Referent / Low / High Tail moment (Ln): 0.67 ± 0.55 / 0.88 ± 0.55* / 1.01 ± 0.56* *ANOVA p-value = 0.006; linear regression model, trend p-value = 0.002, adjusted for age, gender, smoking status, alcohol consumption, duration of employment **By Number of Work Years** <1 (N= 57) / 1–3 (N = 64) / >3 (N = 57)

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr><td>cells/ sample, blinded analysis.</td><td>wood scraps): 0.13 mg/m^3 (0.019−0.252)
Exposure duration: 2.52 yrs</td><td>
<table>
<tr><td>Tail moment (Ln)</td><td>0.76 ± 0.56</td><td>0.73 ± 0.59</td><td>0.99 ± 0.52</td></tr>
</table>
*ANOVA p-value = 0.131; trend p-value = 0.059, Adjusted for age, gender, smoking status, alcohol consumption, and formaldehyde levels
</td></tr>
<tr><td>Gomaa et al. (2012)
Egypt
Prevalence study
Population: 30 workers in pathology, histology and anatomy laboratories at a university (30% female, mean age 42.5 yr) compared to 15 referents (46.7% female, mean age 39.3 yr). Source of referent was not described.
Outcome: Comet assay, alkaline conditions according to Singh et al. (1988); tail length & tail moment; blinding not described; analyzed 50 cells per subject</td><td>No formaldehyde measurements; exposure defined by job type

Exposure duration: mean 14.3 yr</td><td>Comparisons of Comet assay results by exposure
<table>
<tr><th></th><th>Unexposed</th><th>Exposed</th></tr>
<tr><td>Tail length (μm)</td><td>12.5 ± 1.5 (7.2−14.7)</td><td>47.3 ± 8.5* (16.5−74.2)</td></tr>
<tr><td>Tail moment</td><td>10.8 ± 1.2 (5.8−13.6)</td><td>56.1 ± 16.5* (11.4−88.1)</td></tr>
</table>
*Student's t-test, p <0.05; Mean value per 50 comets ± SE, distribution in parentheses.

Results comparable between males and females.</td></tr>
<tr><td>Costa et al. (2011)
Portugal
Prevalence study
Population: 48 pathology workers from 5 hospital laboratories, exposed for at least 1 yr (28% female, mean age 40 yr, 21% smokers), compared to 50 unexposed employees matched by age, gender, lifestyle, smoking habits, and work area (25% female, mean age 37 yr, 14% smokers).
Outcome: DNA damage, comet assay, tail length and % tail DNA; alkaline conditions, 100 cells/</td><td>Air sampling in breathing zone; 8-hr TWA derived for each subject.

Concentration: ppm converted to mg/m^3 by EPA.
Mean: 0.53 mg/m^3
Range: (0.05−1.94)

Duration:
Mean: 13.6 yrs
Range: (1−31)</td><td>Comparisons of Comet assay results by exposure
<table>
<tr><th></th><th>Unexposed</th><th>Exposed</th></tr>
<tr><td>Tail length</td><td>42.00 ± 1.6</td><td>54.55 ± 2.02*</td></tr>
<tr><td>% DNA Tail</td><td>8.01 ± 0.64</td><td>11.76 ± 0.74*</td></tr>
</table>
ANOVA, Student's t-test, p <0.05, compared to referent group.
Tail length and % tail DNA did not vary by gender, age, or smoking. Comet assay parameters were not associated with exposure duration.</td></tr>
</table>

Reference and study design	Exposure	Results
subject; analysis blind to exposure		
Jiang et al. (2010) China Prevalence study **Population:** 151 male workers from 2 plywood plants (mean age 27.4 yr, 52.3% smokers) compared to 112 unexposed workers at a machine manufacturer in same town (mean age 28.7 yr, 42.9% smokers). **Outcome:** Peripheral blood lymphocytes, Comet assay, olive tail moment, alkaline conditions; blinded analysis, analyzed > 100 cells/ subject Related reference: Yu et al. (2005) in Chinese	Exposure assessed by job title and personal air monitoring. 4 exposure groups based on 8-hr TWA: 0.135, 0.344, 0.479, 3.141 mg/m^3. Concentration: ppm converted to mg/m^3 by EPA. Mean: 1.02 mg/m^3 Range: (0.1–0.75) Duration: Mean: 2.51 yrs Range: (0.6 – 25)	**Comparison of Comet assay results in peripheral blood lymphocytes by exposure and duration of employment** **Ln tail moment (TM), geometric mean (95% CI)** Referent (n=112): 0.93 (95%CI: 0.78−1.10) 0.135 mg/m^3 (n = 60): 2.85 (95%CI: 2.37−3.43)* 0.344 mg/m^3 (n=35): 3.01 (95%CI: 2.48−3.64)* 0.479 mg/m^3 (n=43): 4.37 (95%CI: 3.78−5.05)* 3.141 mg/m^3 (n=13): 8.86 (95%CI: 6.50−12.07)** *TM compared to referent group, ANOVA, p<0.05; **TM compared to referent and other exposure groups, ANOVA p<0.05 **Tail moment by exposure history (yrs)*** 0.6−1 (n=33): 2.27 (2.91−3.71) 1−2 (n=68): 2.69 (3.50−4.13) 3−25 (n=50): 3.53 (4.11−4.78)** *ANOVA, p = 0.03, adjusted for age, formaldehyde exposure history and concentration, current smoking status, alcohol consumption **Dunnett-Hsu test, compared to 0.6−1 yr subgroup, p = 0.01
Costa et al. (2008) Portugal Prevalence Study **Population:** 30 pathology lab workers (4 hospitals), (70% female, mean age 38 yr, 27% smokers) compared to 30 administrative employees matched by age, gender, lifestyle, smoking habits and work area (63.3% female, mean age 37 yrs, 23% smokers). **Outcome:** Peripheral lymphocytes; blood samples collected 10−11 am; Scored blind to exposure status; Comet assay, tail length, alkaline conditions (pH = 13), 100 cells/ subject	Air sampling in breathing zone, 8-hr TWA derived for each subject Mean: 0.54 mg/m^3 Range: (0.05−1.94) Years employed: Mean ± SD: 11 ± 7 yrs Range: (0.5−27)	**Comparisons of Comet assay results in peripheral blood lymphocytes by exposure** Unexposed: Tail Length 41.85 ± 1.97 Exposed: Tail Length 60.00 ± 2.31* *p <0.05, Student's t-test Tail length was also significantly longer among exposed females compared to males. No difference noted by smoking status. No difference by duration of exposure (data not provided).
Short-term Exposure		

Reference and study design	Exposure	Results
Lin et al. (2013) China Cross-shift change **Population:** 62 plywood workers (17.7% female, mean age 34 yr, 17.7% smokers) assessed in 2011. **Outcome:** Peripheral blood lymphocytes, change over 8-hr shift; Comet assay, olive tail moment, alkaline conditions (pH = 13), blinded analysis, 50 cells/ subject.	Exposure assessed by air sampling and job function. Mean exposure: 0.27 ± 0.20 mg/m^3 Range: 0.012–0.67 mg/m^3	**Comet assay results before and after work-shift** Ln-transformed Tail moment: Before exposure (*n*= 60): 1.47 ± 0.72; After exposure (*n*= 62): 2.30 ± 1.28* * *p* = < 0.001, paired *t*-test Regression coefficients for formaldehyde level, before shift -0.69 (−2.11, 0.73); after shift 3.64 (1.36, 5.92)
Zeller et al. (2011) Germany Controlled human exposure study **Subjects:** 41 healthy volunteers exposed 4 hr/d for 5 d, all male, nonsmokers **Outcome:** peripheral lymphocytes. Comet assay: alkaline conditions (pH 13). Analyzed 100 cells/ subject on coded slides.	12 groups of 2 to 4 persons in a chamber, exposures randomly assigned. Formaldehyde concentrations: 0, 0.37 mg/m^3, with four peaks of 0.74 mg/m^3, 0.49 mg/m^3 with four peaks 0.98 mg/m^3 and 0.67 mg/m^3 and 0.86 mg/m^3, peaks 15 min, 4 15-min exercise sessions during exposure.	**Results of Comet assay in lymphocytes before and after 4-hr exposure (N = 37)** Tail Moment: Before exposure 0.30 ± 0.117; After exposure 0.33 ± 0.118 Tail Intensity: Before exposure 2.28 ± 0.492; After exposure 2.66 ± 0.646* **p* = 0.002, Wilcoxon signed rank test, compared to preexposure level.
DNA Adducts		
Bono et al. (2010) Italy (Prevalence study) **Population:** 20 pathologists from 3 pathology wards who worked in tissue fixation rooms (production rooms) and 20 students and workers from a university's science labs **Outcome:** M_1dG adducts in DNA extracted from whole blood, methods described in van Helden et al. (2009); compared mean log-transformed	Personal sampling over an 8-hr shift in each subject; LOD 0.05 µg/m^3; questionnaire data on job-specific work (work in production room where slides were fixed or other areas) & use of personal protection Mean formaldehyde in production room 0.212 ± 0.047 mg/m^3, other areas 0.0324 ± 0.0061 mg/m^3,	Mean levels M_1dG adducts per 10^8 NNs by exposure group Referent: N 20; Mean ± SE 2.4 ± 0.3 Exposed: N 20; Mean ± SE 5.7 ± 1.3; p-Value 0.045[1] *8-hr TWA* <22 µg/m^3: N 13; Mean ± SE 2.3 ± 0.44 23–66 µg/m^3: N 13; Mean ± SE 2.7 ± 0.55; p-Value 0.775 >66 µg/m^3: N 13; Mean ± SE 7.3 ± 1.9; p-Value 0.018[2] [1] compared to referent. [2] compared to <22 µg/m^3.

Reference and study design	Exposure	Results
M_1dG adducts by exposure tertile or exposure status, using ANCOVA adjusting for sex, age, smoking	referents 0.028 ± 0.0025 mg/m^3	
DNA-Protein Crosslinks		
Prevalence Studies		
Lin et al. (2013) China (Prevalence) **Population:** 96 plywood workers exposed to formaldehyde (13.5% female, mean age 33 yr, 30.2% smokers) compared to referent group (*N*=82) (4% female, mean age 31 yr, 40% smokers). **Outcome:** Peripheral blood lymphocytes: DNA-protein cross-links (DPX), KCl- SDS assay. blinded analysis	Exposure categories by air monitoring and job assignment. Average concentration: High exposure, *N*=38 (making glue): 1.48 mg/m^3 (range 0.914–2.044) Low exposure, *N*=58 (sanding boards, pressing wood scraps with glue at high temp): 0.68 mg/m^3 (range 0.455–0.792) Referent group, N=82 (providing & grinding wood scraps): 0.13 mg/m^3 (range 0.019–0.252) Exposure duration: 2.52 yrs	**DPX levels in peripheral blood lymphocytes by formaldehyde exposure and years of employment** **DPX by Formaldehyde Level** DPX (%): Referent 22.73 ± 21.47; Low 22.53 ± 22.26; High 20.37 ± 20.52 *ANOVA *p*-value = 0.894; trend *p*-value = 0.682, adjusted for age, gender, smoking status, alcohol use and duration of employment **DPX by Number of Work Years** DPX (%): <1 (*N*= 57) 19.34 ± 20.77; 1–3 (*N*= 64) 22.10 ± 20.98; >3 (*N*= 57) 25.06 ± 20.57 ANOVA, [a] *p*-value = 0.577; [b] trend *p*-value = 0.376. [a]adjusted for age, gender, smoking status, alcohol use, formaldehyde exposure levels [b] Calculated using linear regression models with adjustment for age, gender, smoking status, alcohol use and formaldehyde exposure levels.
Shaham et al. (2003) Israel Prevalence study **Population:** 186 workers from 14 hospital pathology departments (mean age 45.8 yr, 68.3% female, 36.6% smokers) compared to 213 administrative workers from the same hospitals (mean age 42.1 yr, 40.4% female, 44.6% smokers). Age distribution, gender, origin (ethnicity), and years of education differed significantly	Field and personal air sampling, sample duration 15 min, multiple times during work-day (# not reported). Concentration Low exposure: 0.49 (range 0.049–0.86) mg/m^3 High exposure: 2.8 (range 0.89–6.9) mg/m^3 Duration: Mean: 15.9 yrs Range: 1–51 yrs	**Comparison of DNA-protein crosslinks by exposure** Mean DPX/ total DNA ± SE: Referent 0.14 ± 0.006; Exposed 0.21 ± 0.006** **p <0.01, adjusted for age, gender, smoking, education and region of origin. **Mean frequency DNA-protein crosslinks by level of exposure** Mean DPX/ total DNA[1]: Referent 0.14; Low 0.19; High 0.20 [1]SE was not provided. Trend by exposure level was not statistically significant.

Reference and study design	Exposure	Results
between the groups but were adjusted for in the analysis. **Outcome:** peripheral blood lymphocytes**.** Mean percent DPX of total DNA in quantity white blood cells, K-SDS method, double blinded.		
Shaham et al. (1997) Israel Prevalence study **Population:** 12 pathology workers (mean age 44 yr) compared to 8 age-matched controls (mean age 41 yr). **Outcome:** Mean percent DPX, K-SDS method, double blinded Related references: Shaham et al. (1996)	Field and personal air sampling, sample duration 15 min, multiple times during work-day (# not reported). Concentration: Mean: NR Range: 3.4–3.8 mg/m^3 Exposure duration mean 13 yrs (range 2–31 yrs)	**Frequency of DPX by Exposure** Unexposed: Mean DPX % 23 ± 7 Exposed: 29 ± 6* *p = 0.03, ANOVA adjusting for smoking status. Years of exposure linearly correlated with DPX levels.
Short-term Studies		
Lin et al. (2013) China Cross-shift change **Population:** 62 plywood workers (17.7% female, mean age 34 yr, 17.7% smokers) assessed in 2011. **Outcome:** Blood lymphocytes: % cross links measured before and after 8-hr shift, blinded analysis.	Air sampling and job function. Mean exposure: 0.27 ± 0.20 mg/m^3 Range: 0.012–0.67 mg/m^3	**DPX frequency before and after work-shift** Before exposure (*n*= 62): DPX (%) 27.22 ± 10.07 After exposure (*n*= 60): 31.68 ± 14.19* ** p = 0.019, paired t-test.* Regression coefficients for formaldehyde level, before shift 1.70 (−17.84, 21.24); after shift −6.04 (−31.23, 19.15).
DNA Repair		
Schlink et al. (1999) Germany **Population:** Anatomy students, Group 1, 41 students from one university course, 3-hr labs, 2 times per wk (43.9% female, ages 21-30 yr, 39% smokers); Group	Personal sampling near breathing zone once per week, sampling period not reported. formaldehyde exposed, Mean ± SD, 0.2 ± 0.05 mg/m^3, 0.14–0.3 mg/m^3	MGMT activity change compared (U-test, paired data) before and after exposure; as well as between exposure groups (Wilcoxon, Mann and Whitney U-test) Mean MGMT activity by exposure group (fmol MGMT/ 10^6 cells) Group 1: N 41; Day 0 133.2; Day 50 131.1[1]; Day > 90 128.2[1] Group 2: N 16; Day > 90 146.9[2]

Reference and study design	Exposure	Results
2, 16 students from a different university course (50% female, ages 21–27 yr, 37.5% smokers), and Referent, 10 unexposed students (60% female, ages 22–44 yr, 30% smokers); no previous formaldehyde exposure **Outcome:** O^6-alkylguanine DNA alkyl-transferase activity in peripheral blood lymphocytes (modification of Klein and Oesch (1990), expressed as fmol MGMT/ 10^6 cells (LOD 1 fmol MGMT/ 10^6 cells), blind to period of sample (before or after); Blood samples collected before 1st class and after days 50 and 111		Referent 10 138.9 [1]*p* >0.05 compared to Day 0. [2]*p* >0.05 compared to referent. MGMT activity did not differ by gender, smoking, allergy status, or alcohol consumption.
Hayes et al. (1997) USA Panel study **Population:** 29 students (with adequate samples) exposed to formaldehyde for 9 wks during embalming course 16 male, 7 females, 6 smokers. Mean duration of embalming 125 min. 15 with previous embalming exposure within previous 90 da **Outcome:** O^6-alkylguanine DNA alkyltransferase activity in peripheral lymphocytes, expressed as pmol AGT/ mg protein (LOD 0.006 pmol AGT/ mg protein), blind to period of sample (before or after); blood samples collected in morning before 1st class and after 9 wks	Personal sampling for 121 of 144 embalmings; Exposure concentration: Mean: 1.72 mg/m^3 Range: (0.18−5.29) mg/m^3 Duration: 9 wks (0.173 yrs) Total number of reported embalmings correlated with estimated cumulative formaldehyde exposure (r = 0.59, p < 0.01).	Individual data pre- and postcourse AGT activity in peripheral blood lymphocytes depicted in graphs by embalming experience during previous 90 d (yes/ no), decreased in 17 students, increased in 6 students (ANOVA adjusting for age, sex and smoking, p < 0.05).

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr><td>Related reference:
Suruda et al. (1993)</td><td></td><td></td></tr>
<tr><td colspan="3">P53 protein levels in blood</td></tr>
<tr><td>Attia et al. (2014)
Egypt
Prevalence study
Population: 40 employees at cosmetic manufacturing company (23% male, mean age 25.8 yrs, 20% smokers) randomly selected, compared to referent (N=20) selected from hospital administrative department with comparable SES & no history of occupational exposure to formaldehyde (35% male, mean age 34 yrs, 15% smokers)
Outcome: Peripheral blood; plasma MDA (commercial kit), plasma p53 (p53 enzyme-linked immunosorbent assay kit). Blinding not stated. Statistical analyses of coded data (blinded assumed). Exposed compared to referent, means (Student’s t-test), correlation between urinary formate and MDA or p53 using linear regression</td><td>Urine formic acid according to Hopner & Knappe, 1974; unclear how to relate urine formic acid levels to air concentrations

Urinary formate
Exposed: 53.4 ± 15.01 mg/L
Referent: 12.7 ± 4.57 mg/L
P <0.05</td><td>Comparison of plasma p53 and plasma MDA concentrations in exposed and referent groups
<table><tr><th></th><th>Referent</th><th>Exposed</th><th>p-Value</th></tr><tr><td>Plasma p53 (U/mL)</td><td>2.78 ± 0.48</td><td>13.34 ± 4.67</td><td><0.05</td></tr><tr><td>Plasma MDA (nmol/ml)</td><td>3.59 ± 0.83</td><td>9.73 ± 2.72</td><td><0.05</td></tr></table>
Correlations in exposed group:
Urinary formate & p53, r=0.91 p <0.001
Urinary formate & MDA, r =0.79, p <0.001
Plasma MDA & plasma p53, r =0.81, p <0.001

Age and gender were not associated with plasma p53, plasma MDA or urinary formate.</td></tr>
<tr><td>Shaham et al. (2003)
Israel
Prevalence study

Population: 186 workers from 14 hospital pathology departments (mean age 42.1 yr, 59.6% male, 36.6% smokers) compared to 213 administrative workers</td><td>Field and personal air sampling, sample duration 15 min, multiple times during work-day (# not reported). Concentration
Low exposure: 0.49 (range 0.049–0.86) mg/m^3</td><td>Comparisons of exposure, serum total p53, serum mutant p53 and DPXs (OR, 95% CI)
<table><tr><th></th><th>Total</th><th>Male</th><th>Female</th></tr><tr><td>Total p53 protein > 150 pg/mL[a]</td><td></td><td></td><td></td></tr><tr><td>Referent</td><td>1.0</td><td>1.0</td><td>1.0</td></tr><tr><td>Exposed</td><td>1.6 (0.8–3.1)</td><td>2.0 (0.9–4.4)</td><td>0.8 (0.2–2.7)</td></tr><tr><td>Total p53 protein > 150 pg/mL[b]</td><td></td><td></td><td></td></tr><tr><td>DPX ≤ 0.187 [b]</td><td>1.0</td><td>1.0</td><td>1.0</td></tr><tr><td>DPX > 0.187</td><td>2.5</td><td>1.9</td><td>2.8</td></tr></table></td></tr>
</table>

Reference and study design	Exposure	Results
from the same hospitals (mean age 45.8 yr, 31.7% male, 44.6% smokers). Age distribution, gender, origin (ethnicity), and years of education differed significantly between the groups but were adjusted for in the analysis. **Outcome:** p53 proteins (wild type and mutant) in serum, p53 quantitative ELISA kit immunoassay, mutant p53 in serum using quantitative ELISA kit immunoassay. Categorical analysis of p53 levels (>pg/mL), exposure groups compared using chi-square test; logistic regression of p53 >150 pg/mL	High exposure: 2.8 (range 0.89−6.9) mg/m^3 Duration: Mean: 15.9 yrs Range: (1−51) yrs	(1.2−5.4) (0.5−7.2) (1.1−7.1) [a]Logistic regression models adjusted for sex, age and smoking. [b]In the exposed group, logistic regression models adjusted for sex, age and smoking. [b]DPX expressed as % of total DNA. Correlations: Total p53 protein and mutant p53 protein, r =0.75, p <0.01 **Proportion p53 > 150 pg/mL among exposed** DPX ≤ 0.187 33.3% DPX > 0.187 55.7% (p <0.01)
Genetic Susceptibility		
Costa et al. (2019); Costa et al. (2015) Portugal Prevalence study **Population:** 84 anatomy pathology workers from 9 hospital laboratories, exposed to formaldehyde for at least 1 yr, compared to 87 non-exposed employees from administrative offices in same geographic area. Exclusions: cancer history, radiation therapy or chemotherapy, surgery with anesthesia or blood transfusion in last year. Exposed and referent similar for mean age 39 years, 77% females, 25% smokers. **Outcome:** Peripheral blood samples,	Exposure assessed via air sampling and deriving an 8-hr TWA for each subject. Exposure concentration: Mean: 0.38 ppm (0.47 mg/m^3) Range: 0.28−0.85 ppm (0.34–1.05 mg/m^3) Exposure duration 12.0 ± 8.2 yrs	Effect modification by genetic polymorphisms on associations of formaldehyde with markers of genotoxicity (mean ratio, 95% CI) Referent: N / MR (95% CI); Exposed: N / MR (95% CI) CYP2E1 rs6413432 (% tDNA) T/T: 53 / 1.00; 51 / 1.61 (1.20−2.16) T/A + A/A: 15 / 0.84 (0.54−1.30); 7 / 0.42 (0.20−0.89) GSTP1 rs1695 (CSAs) Ile/Ile: 32 / 1.00; 37 / 5.43 (2.04−14.46) Ile/Val + Val/Val: 55 / 1.79 (1.14−7.94); 47 / 0.26 (0.97−3.27) XRCC1 rs1799782 (% tDNA) Arg/Arg: 67 / 1.00; 53 / 1.46 (1.10-1.93) Arg/Trp: 2 / 0.19 (0.06−0.57); 6 / 4.93 (1.33−18.32) PARP1 rs1136410 (Multiabberrant cells)

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr><td>coded, analyses blinded to exposure status. Differences in genotype distribution evaluated using Pearson's chi-square test, effect modification by genotype in regression models of exposure on ln % tDNA (comet assay) and chromosome aberrations, CYP2E1 rs6413432, GSTM1 deletion, GSTT1 deletion, GSTP1 rs1695, XRCC1 rs1799782, XRCC1 rs25487, PARP1 rs1136410, MUTYH rs3219489, XRCC3 rs861539</td><td></td><td>
<table>
<tr><td>Val/Val</td><td>60</td><td>1.00</td><td>50</td><td>5.97 (2.34–15.25)</td></tr>
<tr><td>Val/Ala</td><td>8</td><td>3.00 (0.55–16.4)</td><td>9</td><td>0.09 (0.01–0.95)</td></tr>
</table>
Regression models adjusted for age, gender, smoking habit, and fruit consumption.

Micronuclei frequency (%/1,000 cells) by genetic polymorphisms in formaldehyde exposed and unexposed workers
<table>
<tr><td></td><td colspan="2">Controls</td><td colspan="2">Exposed</td></tr>
<tr><td>Gene site</td><td>N</td><td>Mean ± SE</td><td>N</td><td>Mean (SE)</td></tr>
<tr><td>CYP2E1 rs6413432 BNbud</td><td></td><td></td><td></td><td></td></tr>
<tr><td>T/T</td><td>53</td><td>0.36 ± 0.077</td><td>51</td><td>0.80 ± 0.12</td></tr>
<tr><td>T/A + A/A</td><td>15</td><td>0.20 ± 0.11</td><td>7</td><td>1.57 ± 0.20*</td></tr>
<tr><td>GSTP1 rs1695 MNB</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Ile/Ile</td><td>28</td><td>0.14 ± 0.07</td><td>29</td><td>0.45 ± 0.11</td></tr>
<tr><td>Ile/Val + Val/Val</td><td>41</td><td>0.20 ± 0.07</td><td>33</td><td>0.82 ± 0.15*</td></tr>
<tr><td>FANCA rs7190823 MNL</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Thr/Thr</td><td>9</td><td>2.33 ± 0.93</td><td>12</td><td>2.33 ± 0.57</td></tr>
<tr><td>Thr/Ala + Ala/Ala</td><td>77</td><td>2.84 ± 0.32</td><td>70</td><td>4.74 ± 0.44*</td></tr>
</table>
* p-values CYP2E1 rs6413432 A variant, 0.022; GSTP1 rs1695 Val variant 0.05; FANCA rs7190823 Ala variant 0.019
</td></tr>
<tr><td>Ladeira et al. (2013)
Portugal
Prevalence study
Population: 54 hospital workers in histopathology labs compared to 82 administrative staff.
Outcome: Genotyping XRCC3 Met241Thr, ADH5 Val309Ile, ADH5 Asp353Glu; associations of polymorphism with mean micronuclei, nucleoplasmic bridges and nuclear buds in lymphocytes and buccal</td><td>Personal air sampling, 6-8 hours, estimated 8-hr TWA
Exposure conc.: Mean TWA 8 hr 0.2 ± 0.14 mg/m^3
Mean ceiling value: 1.4 ± 0.91 mg/m^3, range 0.22–3.6 mg/m^3

Exposure duration: 14.5 (1–33) yrs</td><td>
Frequency of micronuclei and nuclear buds (mean ± SE) in lymphocytes by exposure and genotype (number in parentheses)
<table>
<tr><td>Endpoint</td><td colspan="3">Genotypes</td></tr>
<tr><td>MN</td><td colspan="3"></td></tr>
<tr><td></td><td colspan="3">XRCC3</td></tr>
<tr><td></td><td>Met/Met</td><td>Thr/Met</td><td>Thr/Thr</td></tr>
<tr><td>Exposed (p=0.372)</td><td>2.92 ± 0.93 (13)</td><td>5.05 ± 0.98 (22)</td><td>3.53 ± 0.80 (19)</td></tr>
<tr><td>Referent (p=0.621)</td><td>1.15 ± 0.46 (20)</td><td>0.70 ±0.30 (27)</td><td>0.74 ± 0.23 (35)</td></tr>
<tr><td></td><td colspan="3">ADH5</td></tr>
<tr><td></td><td>Val/Val</td><td>Val/Ile</td><td></td></tr>
<tr><td>Exposed (p=0.024)</td><td>2.57 ± 0.65 (21)</td><td>4.91 ± 0.75 (33)</td><td></td></tr>
</table>
</td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Exposure</th><th>Results</th></tr>
<tr><td>cells within exposed and referent groups, Kruskal-Wallis test

Related references:
Ladeira et al. (2011)</td><td></td><td>
<table>
<tr><td>Referent (p=0.176)</td><td>0.97 ± 0.28 (29)</td><td>0.75 ± 0.23 (53)</td><td></td></tr>
<tr><td></td><td colspan="3">ADH5</td></tr>
<tr><td></td><td>Asp/Asp</td><td>Asp/Glu</td><td></td></tr>
<tr><td>Exposed (p=0.70</td><td>4.08 ± 0.91 (24)</td><td>3.93 ± 0.67 (30)</td><td></td></tr>
<tr><td>Referent (p=0.211)</td><td>0.86 ± 0.23 (35)</td><td>0.81 ± 0.26 (47)</td><td></td></tr>
<tr><td>NBUD</td><td colspan="3"></td></tr>
<tr><td></td><td colspan="3">XRCC3</td></tr>
<tr><td></td><td>Met/Met</td><td>Thr/Met</td><td>Thr/Thr</td></tr>
<tr><td>Exposed (p=0.002)</td><td>0.38 ± 0.18 (13)</td><td>1.5 ± 0.33 (22)</td><td>0.21 ± 0.12 (19)</td></tr>
<tr><td>Referent (p=0.045)</td><td>0.2 ± 0.09 (20)</td><td>0.04 ± 0.04 (27)</td><td>0.03 ± 0.29 (35)</td></tr>
<tr><td></td><td colspan="3">ADH5</td></tr>
<tr><td></td><td>Val/Val</td><td>Val/Ile</td><td></td></tr>
<tr><td>Exposed (p=0.274)</td><td>0.62 ± 0.28 (21)</td><td>0.88 ± 0.21 (33)</td><td></td></tr>
<tr><td>Referent (p=0.061)</td><td>0.00 ± 0.0 (29)</td><td>0.11 ± 0.04 (53)</td><td></td></tr>
<tr><td></td><td colspan="3">ADH5</td></tr>
<tr><td></td><td>Asp/Asp</td><td>Asp/Glu</td><td></td></tr>
<tr><td>Exposed (p=0.74)</td><td>0.71 ± 0.23 (24)</td><td>0.83 ± 0.25 (30)</td><td></td></tr>
<tr><td>Referent (p=0.633)</td><td>0.06 ± 0.04 (35)</td><td>0.09 ± 0.04 (47)</td><td></td></tr>
</table>

No differences noted for nucleoplasmic bridges or micronuclei in buccal cells (data provided in article)</td></tr>
<tr><td>Santovito et al. (2011)
Italy
Prevalence study
Population: 20 pathology workers (mean age 45.7 yr) compared to 16 workers from the same hospital (mean age 42.1 yr); similar age and gender distribution. All subjects were non-smokers and had not consumed alcohol in 1 yr.
Outcome: Genotypes GSTT, GSTM; associations of polymorphisms with CA per cell and % of cells with aberrations within</td><td>Exposure conc:
Personal air sampling, 8-hr duration.
Referent: Mean: 0.036 ± 0.002 mg/m^3
Pathologists: Mean: 0.073 ± 0.013 mg/m^3

Exposure duration:
Mean: 13 yrs
Range: 2–27 yrs</td><td>Frequency of chromosomal aberrations per cell (mean ± SE) in lymphocytes by exposure and genotype (number in parentheses)
<table>
<tr><th></th><th>Exposed</th><th>Referent</th></tr>
<tr><td>GSTT-pos</td><td>0.028 ± 0.003 (16)</td><td>0.01 ± 0.004 (12)</td></tr>
<tr><td>GSTT-null</td><td>0.04 ± 0.015 (4)</td><td>0.013 ±0.009 (4)</td></tr>
<tr><td>GSTM-pos</td><td>0.031 ± 0.004 (17)</td><td>0.01 ± 0.004 (10)</td></tr>
<tr><td>GSTM-null</td><td>0.023 ± 0.003 (3)</td><td>0.012 ± 0.008 (6)</td></tr>
</table>

No differences also were found for the % of cells with chromosomal aberrations (data provided in article).</td></tr>
</table>

Reference and study design	Exposure	Results
exposed and referent groups; generalized linear models with Poisson distribution errors adjusted for gender and age		
Jiang et al. (2010) China Prevalence **Population:** 151 male workers from 2 plywood plants (mean age 27.4 yr, 52.3% smokers) compared to 112 unexposed workers at a machine manufacturer in same town (mean age 28.7 yr, 42.9% smokers). **Outcome:** genotypes GSTM1, GSTT1, GSTP1; associations with olive TM and CBMN frequency within exposed and referent; ANCOVA adjusted for age, smoking and alcohol	Exposure assessed by job title and personal air monitoring. Exposure concentration ppm converted to mg/m^3 by EPA. 1.08 mg/m^3, range 0.1−7.75 mg/m^3 Duration: Mean 2.51 yrs Range: (0.5−25) yrs	Frequency of olive TM (geometric mean (95% CI) in lymphocytes by exposure and genotype (number in parentheses) Exposed / Referent GSTM1-pos: 3.27 (2.83−3.78) 74) / 1.01 (0.77−1.32) (46) GSTM1-null: 3.86 (3.31−4.5) (77) / 0.87 (0.69−1.1) (66) *P* =0.07 / *P* =0.43 GSTT1-pos: 3.72 (3.26−4.25) (83) / 1.04 (0.82−1.31) (63) GSTT1-null: 3.36 (2.83−3.99) (68) / 0.8 (0.61−1.04) 49) *P* =0.47 / *P* =0.11 GSTP1-Ile/Ile: 3.64 (3.19−4.16) (90) / 0.96 (0.74−1.23) (58) GSTP1 Val pos: 3.43 (2.87−4.1) (61) / 0.89 (0.7−1.14) (54) *P* = 0.49 / *P* = 0.83 Frequency of ln CBMN (mean ± SD) in lymphocytes by exposure and genotype (number in parentheses) Exposed / Referent GSTM1-pos: 5.57 ± 3.45 (74) / 2.91 ± 1.5 (46) GSTM1-null: 5.5 ± 3.32 (77) / 2.5 ± 1.15 (66) *P* = 0.84 / *P* = 0.18 GSTT1-pos: 5.59 ± 3.51 (83) / 2.75 ± 1.41 (63) GSTT1-null: 5.46 ± 3.22 (68) / 2.57 ± 1.19 (49) *P* = 0.70 / *P* = 0.47 GSTP1-Ile/Ile: 5.01 ± 2.98 (90) / 2.79 ± 1.36 (58) GSTP1 Val pos: 6.32 ± 3.78 (61) / 2.54 ± 1.27 (54) *P* = 0.05 / *P* =0.26

ADH, alcohol dehydrogenase; AGT, O^6-alkylguanine-DNA alkyltransferase; ANOVA, analysis of variance; C–, centromere negative; C+, centromere positive; CA, chromosomal aberration; CB-MN or CBMN, cytokinesis block-micronucleus; CFU-GM, colony forming unit-granulocyte/macrophage; CI, class interval; CSA, chromosome-type aberration; CSG, centromere separation general; CTA, chromatid-type aberration; DAPI, diamidinophenylindole;

DPX, DNA-protein crosslink; EA, ethyl acetate; ELISA, enzyme-linked immunosorbent assay; FISH, fluorescence *in situ* hybridization; GST, glutathione S-transferase; HCHO, formaldehyde; HF, high frequency; IRR, incidence rate ratio; K-SDS/KCl-SDS, potassium chloride-sodium dodecyl sulfate; LOD, level of detection; LTR, lymphocyte transformation rate; M_1dG, malondialdehyde-deoxyguanosine; MAK, maximum permissible concentration (German); MDA, malondialdehyde; MGMT, O^6-methylguanine methyl transferase; MN, micronucleus; MR, mean ratio; NSM, number of scored metaphases; OR, odds ratio; PARP, poly (ADP-ribose) polymerase; PCD, premature centrosome division; PI, proliferation index; SCE, sister chromatid exchange; SD, standard deviation; SE, standard error; SEM, standard error of the mean; tDNA, tail DNA; TWA, total weighted average; XRCC, X-ray repair cross complementing.

A.4.7. Supporting Material for Genotoxicity

Literature Search Methods for Genotoxic Endpoints

A systematic evaluation of the literature database on studies examining potential genotoxic endpoints in relation to formaldehyde exposure was not conducted. However, a consistent set of search terms was used, initially in September 2012, with regular updates as described elsewhere. These terms were intended to inform the broader topic of mode of action for either respiratory tract or lymphohematopoietic cancers and the retrieved citations were screened for studies on genotoxic endpoints. The search strings used in specific databases are shown in Table A-25. Additional search strategies included:

- Review of reference lists in identified articles, and
- Review of reference lists in the 2010 draft Toxicological Review for Formaldehyde (U.S. EPA, 2010).

Table A-25. Summary of search terms for cancer mechanisms

Mechanisms for Repiratory Tract Cancers - Pubmed	
1	(formaldehyde[tiab] OR formaldehyde[mh])
2	AND (nose[tiab] OR nasal[tiab] OR nasopharynx[tiab] OR nasopharyngeal[tiab] OR respiratory[tiab] OR bronchial[tiab] OR "upper respiratory"[tiab] OR mucociliary[tiab] OR mononuclear[tiab] OR "nasal mucosa"[tiab] OR "human bronchial"[tiab] OR "nasal cavity"[tiab] OR trachea[tiab] OR "oral mucosa"[tiab] OR lymphoblasts[tiab] OR "endothelial cells"[tiab] OR "respiratory tract"[tiab] OR olfactory[tiab] OR "nasal epithelia"[tiab] OR "nasal turbinates"[tiab] OR "nose"[mh] OR "nasopharynx"[mh] OR "trachea"[mh] OR "smell"[mh])
3	AND (tumor[tiab] OR carcinoma[tiab] OR cancer[tiab] OR neoplastic[tiab] OR cytotoxic[tiab] OR cytotoxicity[tiab] OR proliferation[tiab] OR "cell proliferation"[tiab] OR immunosuppression[tiab] OR immune[tiab] OR genotoxicity[tiab] OR genotoxic[tiab] OR mutation[tiab] OR mutagenic[tiab] OR epigenomic[tiab] OR epigenetic[tiab] OR microRNA[tiab] OR "micro RNA"[tiab] OR methylation[tiab] OR "chromosome aberration"[tiab] OR "chromosomal aberration"[tiab] OR micronuclei[tiab] OR MN[tiab] OR micronucleus[tiab] OR "sister chromatid exchange"[tiab] OR SCE[tiab] OR "single strand break"[tiab] OR SSB[tiab] OR glutathione[tiab] OR oxidation[tiab] OR "oxidative damage"[tiab] OR inflammation[tiab] OR "DNA-protein crosslink"[tiab] OR DPX[tiab] OR "DNA adduct"[tiab] OR clastogen[tiab] OR clastogenicity[tiab] OR promotion[tiab] OR promoter[tiab] OR "DNA repair"[tiab] OR "immune activation"[tiab] OR phagocyte[tiab] OR macrophages[tiab] OR cytogenetic[tiab] OR "regenerative cell proliferation"[tiab] OR mutagenesis[tiab] OR "DNA-protein crosslinks"[tiab] OR "respiratory cancer"[tiab] OR "nasal cancer"[tiab] OR "immune function"[tiab] OR "immune biomarkers"[tiab] OR "respiratory disease"[tiab] OR DPC[tiab] OR DPX[tiab] OR "DNA damage"[tiab] OR irritation[tiab] OR bronchitis[tiab] OR "regenerative hyperplasia"[tiab] OR toxicological[tiab] OR adenomas[tiab] OR rhinitis[tiab] OR dysplasia[tiab] OR metaplasia[tiab] OR inhalation[tiab] OR carcinogen[tiab] OR "chromosomal damages"[tiab] OR "nasal carcinoma"[tiab] OR toxicology[tiab] OR toxicity[tiab] OR "DNA-DNA cross-link"[tiab] OR "respiratory epithelium"[tiab] OR SCC[tiab] OR "pathological changes"[tiab] OR "histopathological nasal changes"[tiab] OR cilia[tiab] OR "nasal lesions"[tiab] OR "protein oxidation"[tiab] OR "cellular immunity"[tiab] OR autoantibodies[tiab] OR tumour[tiab] OR "cell damage"[tiab] OR "neoplasms"[mh] OR "carcinoma"[mh] OR "immunosuppression"[mh] OR "immune tolerance"[mh] OR "mutation"[mh] OR "epigenomics"[mh] OR "methylation"[mh] OR "glutathione"[mh] OR "inflammation"[mh] OR "phagocytes"[mh] OR "macrophages"[mh] OR "cytogenetics"[mh] OR "mutagenesis"[mh] OR "nose neoplasms"[mh] OR "bronchitis"[mh] OR

Mechanisms for Repiratory Tract Cancers - Pubmed	
	"adenoma"[mh] OR "rhinitis"[mh] OR "metaplasia"[mh] OR "inhalation"[mh] OR "carcinogens"[mh] OR "toxicology"[mh] OR "toxicity"[Subheading] OR "cilia"[mh] OR "autoantibodies"[mh] OR "immune system phenomena"[mh] OR "mutagens"[mh] OR "Cytotoxicity, Immunologic"[mh] OR "Cell Proliferation"[mh] OR "MicroRNAs"[mh] OR "Chromosome Aberrations"[mh] OR "Sister Chromatid Exchange"[mh] OR "DNA Breaks, Single-Stranded"[mh] OR "DNA Adducts"[mh] OR "Promoter Regions, Genetic"[mh] OR "DNA Repair"[mh] OR "Respiratory Tract Diseases"[mh] OR "DNA Damage"[mh] OR "Respiratory Mucosa"[mh] OR "Immunity, Cellular"[mh])
4	NOT ("formalin test"[tiab] OR "formaldehyde fixation"[tiab] OR "formalin fixed"[tiab] OR "formaldehyde fixed"[tiab] OR formalin-induced[tiab] OR formaldehyde-induced[tiab])
Mechanisms of LHP Cancers - Pubmed	
1	(formaldehyde[tiab] OR formaldehyde[mh])
2	AND (blood[tiab] OR lymphocytes[tiab] OR "bone marrow"[tiab] OR hematopoietic[tiab] OR "hematopoietic stem cells"[tiab] OR leukocytes[tiab] OR "white blood cell"[tiab] OR "NK cell"[tiab] OR "natural killer cell"[tiab] OR b-lymphocyte[tiab] OR b-cell[tiab] OR t-lymphocyte[tiab] OR t-cell[tiab] OR leukemia[tiab] OR lymphoma[tiab] OR myeloid[tiab] OR serum[tiab] OR albumin[tiab] OR adduct[tiab] OR genotoxic[tiab] OR aneuploidy[tiab] OR pancytopenia[tiab] OR epigenomics[tiab] OR epigenetic[tiab] OR microRNA[tiab] OR "micro rna"[tiab] OR methylation[tiab] OR "chromosome aberration"[tiab] OR "chromosomal aberration"[tiab] OR micronucleus[tiab] OR "sister chromatid exchange"[tiab] OR glutathione[tiab] OR oxidation[tiab] OR "oxidative damage"[tiab] OR inflammation[tiab] OR dna-protein-crosslink[tiab] OR "dna adduct"[tiab] OR "immune activation"[tiab] OR "blood"[Subheading] OR "blood"[mh] OR "lymphocytes"[mh] OR "lymphocyte count"[mh] OR "bone marrow"[mh] OR "hematopoietic system"[mh] OR "hematopoietic stem cells"[mh] OR "leukocytes"[mh] OR "leukocyte count"[mh] OR "leukocytes"[mh] OR "killer cells, natural"[mh] OR "killer cells, natural"[mh] OR "b-lymphocytes"[mh] OR "b-lymphocytes"[mh] OR "t-lymphocytes"[mh] OR "t-lymphocytes"[mh] OR "leukemia"[mh] OR "lymphoma"[mh] OR "serum"[mh] OR "albumins"[mh] OR "aneuploidy"[mh] OR "pancytopenia"[mh] OR "epigenomics"[mh] OR "epigenomics"[mh] OR "micrornas"[mh] OR "micrornas"[mh] OR "methylation"[mh] OR "chromosome aberrations"[mh] OR "chromosome aberrations"[mh] OR "sister chromatid exchange"[mh] OR "glutathione"[mh] OR "inflammation"[mh] OR "dna adducts"[mh])
3	NOT ("formalin test"[tiab] OR "formaldehyde fixation"[tiab] OR "formalin fixed"[tiab] OR "formaldehyde fixed"[tiab] OR formalin-induced[tiab] OR formaldehyde-induced[tiab])
Mechanisms of Respiratory Tract Cancers - WoS	
1	Formaldehyde (Title only)
2	AND (nose OR nasal OR nasopharynx OR nasopharyngeal OR respiratory OR bronchial OR upper-respiratory OR mucociliary OR mononuclear OR nasal-mucosa OR human-bronchial OR nasal-cavity OR trachea OR oral-mucosa OR lymphoblasts OR endothelial-cells OR respiratory-tract OR olfactory OR nasal-epithelia OR nasal-turbinates)
3	AND (tumor OR carcinoma OR cancer OR neoplastic OR cytotoxic OR cytotoxicity OR proliferation OR immunosuppression OR immune OR genotoxicity OR genotoxic OR mutation OR mutagenic OR epigenomic OR epigenetic OR microRNA OR micro-RNA OR methylation OR chromosome-aberration OR chromosomal-aberration OR micronuclei OR MN OR micronucleus OR sister-chromatid-exchange OR SCE OR single-strand-break OR SSB OR glutathione OR oxidation OR oxidative-damage OR inflammation OR DNA-protein-crosslink OR DPX OR DNA-adduct OR clastogen OR clastogenicity OR promotion OR promoter OR DNA-repair OR immune-activation-phagocyte OR macrophages OR cytogenetic OR regenerative-cell-proliferation OR mutagenesis OR DNA-protein-crosslinks OR respiratory-cancer OR nasal-cancer OR immune-function OR immune-biomarkers OR respiratory-disease OR DPC OR DPX OR DNA-damage OR irritation OR bronchitis OR regenerative-hyperplasia OR toxicological OR adenomas OR rhinitis OR dysplasia OR metaplasia OR inhalation OR carcinogen OR chromosomal-damages OR bronchitis OR nasal-carcinoma OR toxicology OR toxicity OR DNA-DNA-cross-link OR respiratory-epithelium OR SCC OR pathological-changes OR histopathological-nasal-changes OR cilia OR nasal-lesions OR protein-oxidation OR cellular-immunity OR autoantibodies OR tumour OR cell-damage)

Mechanisms for Repiratory Tract Cancers - Pubmed	
4	NOT (formalin-test OR formaldehyde-fixation OR formalin-fixed OR formaldehyde-fixed OR formalin-induced OR formaldehyde-induced)
Mechanisms of LHP Cancers - WoS	
1	Formaldehyde (Title only)
2	AND (blood OR lymphocytes OR bone-marrow OR hematopoietic OR hematopoietic-stem-cells OR leukocytes OR white-blood-cell OR NK-cell OR natural-killer-cell OR b-lymphocyte OR b-cell OR t-lymphocyte OR t-cell OR leukemia OR lymphoma OR myeloid OR serum OR albumin OR adduct OR genotoxic OR aneuploidy OR pancytopenia OR epigenomics OR epigenetic OR microRNA OR micro-rna OR methylation OR chromosome-aberration OR chromosomal-aberration OR micronucleus OR sister-chromatid-exchange OR glutathione OR oxidation OR oxidative-damage OR inflammation OR dna-protein-crosslink OR dna-adduct OR immune-activation)
3	NOT (formalin-test OR formaldehyde-fixation OR formalin-fixed OR formaldehyde-fixed OR formalin-induced OR formaldehyde-induced)

Study Evaluations of Epidemiological Studies of Genotoxic Endpoints

Epidemiological studies examining genotoxic endpoints were evaluated for potential bias and other issues using the same domains as were assessed for studies in other health effects categories (see Table A-26). Rather than confidence conclusions of low, medium or high, an overall conclusion of "no obvious bias" was used if no concerns were identified. For studies with a potential bias identified, the potential bias or issue was summarized in the comment row. For each assay (e.g., chromosomal aberrations, CBMN, Comet assay), factors related to assay methods that could affect the endpoint values were identified using published reviews from collaborations that compared assay methods across epidemiological studies (Fenech, 2020; Møller et al., 2020; Bonassi et al., 2011; Fenech et al., 2011; Valverde and Rojas, 2009; Bonassi et al., 2005). Such factors included sample collection and processing flows, whether sample processing and analysis was blinded to exposure status, cell culture details, details of scoring (number of scorers, criteria, staining, number of cells scored). An appropriate citation to a standardized assay protocol was considered acceptable. These reviews noted that assay results have been found to vary by age, gender and smoking status; studies that did not report assessing confounding by these factors were identified. In the study evaluation table for each study, row cells have been given a grey fill for evaluation domains with identified concerns about methods. Study evaluation concerns are discussed in the syntheses of genotoxic endpoints if they may explain observed heterogeneity in study results.

Table A-26. Evaluation of genotoxicity endpoints in epidemiology studies of formaldehyde exposure

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Aglan and Mansour (2018) (Egypt) Hair stylists	Passive air sampling (Umex-100) at fixed position in breathing zone, 15-min samples during hair straightening process; 15-min TWA Group 1 (work duration < 5 yrs): 1.68 ± 0.27 ppm Group 2 (work duration > 5 yrs): 1.83 ± 0.16 ppm	Blood collected at end of 8-hr shift on day hair straightening occurred, processed within 6 hrs. Cytokinesis block micronucleus test in lymphocytes Maffei et al. (2002). Replicate cultures for each sample, incubated 72 hrs, cytochalasin-B added for the last 28 hrs. 1,000 binucleated cells examined per person. 2,000 binucleated cells from coded slides (1,000 from each replicate culture), scored using criteria by Fenech et al. (2003). MN frequency % altered cells. MN in exfoliated buccal cells. Cheeks scraped with wooden spatula, fixed in 3:1	60 female hairstylists selected between June 2015 and September 2016, aged 20–36 years with comparable work hours, number of clients, usual tasks included hair straightening and no gaps in employment. Excluded subjects with chronic disease and /or regular medications, family history of cancer, recurrent abortions, smoking or pregnancy. Comparison group was 60 healthy female hair stylists who did not straighten hair "matched age, residency,	Exposed participants were comparable for work tasks, number of clients and work duration. Only nonsmokers were included, and all were female. Exposed and unexposed were "matched" for age, residency, nutritional habits and SES.	Comparisons between unexposed, group 1 and group 2 using Kruskal Wallis test for nonnormally distributed variables (MNL and MNB) and least significant difference. Comparisons were across duration (greater or less than 5 yrs) and 15-min TWA concentrations were higher in Group 2 (p = 0.03, t test).	Unexposed n = 60 Group 1 n = 31 Group 2 n = 29	Reporting deficiencies result in some concern about potential for selection bias. Comparisons were for duration of exposure (greater or less than 5 yrs) and 15-min TWA concentrations also were statistically different in these groups.

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		methanol/acetic acid and dropped onto slides. Air dried slides stained with Feulgen/Fast Green, examined at 400× according to Tolbert et al. (1991). Analyzed independently by 2 people, 1,500 cells scored per person using criteria by Sarto et al. (1987) % altered cells.	nutritional habits, and socio-economic standard." Participation rates not reported. No data provided to confirm asserted comparability between exposed and referents.				
Attia et al. (2014) (Egypt) Cosmetic manufacture	Urine formic acid according to Hopner and Knappe (1974); unclear how to relate urine formic acid levels to air concentrations	Peripheral blood; plasma MDA (commercial kit), plasma p53 (p53 enzyme-linked immunosorbent assay kit. Blinding not stated, but likely minimal bias because interpretation not required	40 employees at company randomly selected compared to referent (*N* = 20) selected from hospital administrative department with comparable gender and SES & no history of occupational exposure to formaldehyde	Age differed between exposed and referent, but age and gender were not associated with formate levels, MDA levels, or p53 levels	Analyses of coded data (blinded assumed) Exposed compared to referent, means (Student's *t*-test), correlation between urinary formate and MDA or p53 using linear regression	Exposed *n* = 40, referent *n* = 20	No obvious bias

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Aydın et al. (2013) (Turkey) Medium density fiberboard plants (prevalence study)	24 area samples in workplaces; personal samples in breathing zone over 8-hr period. 8-hr TWA calculated	Peripheral blood lymphocytes; samples processed within 6 hr, comet assay, tail intensity, tail moment, and tail migration, alkaline conditions, Singh et al. (1988), cells lysed >1 hr, electrophoresis 20 min, 100 cells/ subject (2 replicates), image analysis software. Blinding not stated	Selection & recruitment of exposed and referent not described. Participation rates not reported. 46 male workers compared to 46 nonexposed males in same area (administrative government offices and maintenance services)	Exposed and referent comparable with respect to age, sex, lifestyle, and smoking habit. No history of occupational exposure to formaldehyde or other chemicals	ANOVA or Kruskal-Wallis H test depending on test for normality; presented mean & SD by exposure group, stratified by smoking status Results of test for normality were not reported, comet assay endpoints were not ln-transformed	Exposed *N* = 46 Referent *N* = 46	No obvious bias
Ballarin et al. (1992) (Italy) Plywood factory	Personal samplers, Sampling in warehouse (*N* = 3) shearing-press (*N* = 8) & sawmill (*N* = 1), sampled formaldehyde and wood dust Calculated 8-hr TWA, reference for measurements (NIOSH, 1977).	Nasal respiratory mucosa cells, cell collection using endocervical brush, smeared onto previously coded slides, stain Feulgen's reaction plus Fast Green, MN, analysis blinded by one reader for cytogenetic, 6,000 cells/subject, scoring criteria Sarto et al. (1987)	Selection & recruitment of exposed and referent not described. Participation rates not reported. Referent from different source population: university or hospital clerks; excluded heavy drinkers	All nonsmokers, matched to referent for age and sex	Differences analyzed using Mann-Whitney test	Exposed *n* = 15; Referent *n* = 15	Small sample numbers; no obvious bias

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Bauchinger and Schmid (1985) (Germany) Papermaking	Exposure assessment based on air monitoring and job-function. Sampling design and duration was not described.	Peripheral lymphocytes, CA/ cell (scored 500 cells/subject), Giemsa staining; SCE/cell (scored 50/subject) analyzed using coded slides	Selection & recruitment of exposed and referent not described. Participation rates not reported. Exposed and referent worked at same factory	All male, Comparable for age, more smokers among referent; no previous radiation history or exposure to other industrial chemicals	Mann-Whitney rank U test to compare groups, SCE analysis stratified by smoking	Exposed *N* = 20; Referent *N* = 20	Possible bias toward null because no adjustment for smoking in CA analysis
Bono et al. (2010) (Italy) Pathology labs	Personal sampling over an 8-hour shift in each subject; LOD 0.05 µg/m^3; questionnaire data on job-specific work (work in production room where slides were fixed or other areas) & use of personal protection	M_1dG adducts in DNA extracted from whole blood, methods described in van Helden et al. (2009); evaluated in 20 out of 40 exposed and 20 out of 32 referent workers (selection criteria were not described)	Selection & recruitment of exposed and referent not described. Participation rates not reported. Recruited workers from 3 pathology labs and workers & students from a university lab with no exposure to formaldehyde	Mean formaldehyde levels varied by age, smoking, and exposure status (referent, work in production room, work in other areas); confounding assessed in analysis	Formaldehyde exposure tertiles based on 8-hr average formaldehyde concentration, compared mean log-transformed M_1dG adducts by exposure tertile or exposure status, using ANCOVA adjusting for sex, age, smoking; evaluated multiple comparisons using Dunnett tests	Exposed *N* = 20 Referent *N* = 20	No obvious bias; small sample size especially for analysis of effect modification by smoking
Bouraoui et al. (2013) (Tunisia)	Area sample in macroscopic room, diffuse radical samplers containing 2,4-dinitrophenyl-	Cytokinesis-blocked MN assay in peripheral lymphocytes in combination with FISH using all-	Recruitment and selection not described. Participation rates not reported. Excluded x-ray	Comparison groups were similar for potential confounders	Multivariate regression of genotoxic markers with possible confounders excluding smokers;	Exposed *n* = 31 Referent *n* = 31	No obvious bias

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Anatomy/ pathology lab in hospital	hydrazine, 24-hr duration, 3 samplings.	chromosome centromeric probe Sari-Minodier et al. (2002); cultured 72 hr, smeared onto slides, stain 5% Giemsa, 2,000 binucleated cells scored/subject, criteria Fenech (2000) blinding not described.	history during previous 6 mos, use of drugs		age and gender were associated but exposure groups were comparable		
Burgaz et al. (2001) (Turkey) Anatomy/ pathology departments in hospital & university	Stationary area measurements; number of samples and duration not reported	Nasal respiratory mucosal cells; collected using endocervical brush, cells smeared onto previously coded slides, stain Feulgen's reaction plus Fast Green, MN, 3,000 cells/ subject counted, scoring criteria Sarto et al. (1987) and Tolbert et al. (1992)	Recruitment and selection not described. Referents worked in same hospital & university	Higher proportion of females in exposed (referent was only male), slightly older individuals, and smokers (and heavy smokers) in referent. Analyses stratified by smoking. Stated that referents had no occupational exposure to genotoxic agents.	Comparison of means using nonparametric methods, two-tailed tests, stratified by smoking; correlation using Spearman's test	Exposed *n* = 23, Referent *n* = 25	Possible bias to null because of age in referent
Burgaz et al. (2002) (Turkey) Anatomy/ pathology departments in	Stationary area measurements; number of samples and	Buccal mucosal cells; cells collected with wooden spatula, smeared onto slides, stain Feulgen's	Recruitment and selection not described. Referents worked	Higher proportion of females (referent was only male), and smokers in referent. Age	Comparison of means using nonparametric methods (Mann-Whitney test), two-	Exposed *n* = 28, Referent *n* = 18	No obvious bias

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
hospital & university Possible overlap with Burgaz et al. (2001)	duration not reported	reaction plus Fast Green, MN, 3,000 cells/ subject counted, coded slides, scoring criteria Sarto et al. (1987) and Tolbert et al. (1992)	in same hospital & university	comparable. Stated that referents had no occupational exposure to genotoxic agents;	tailed tests, correlation using Spearman's test Multifactorial ANOVA adjusting for smoking, exposure and gender and age		
Costa et al. (2008) (Portugal) Hospital pathology laboratories (n = 4) (prevalence)	Samples in breathing zone, NIOSH method #3500. Sampling duration, sample number were not given. 8-hr TWA calculated for each worker	Peripheral lymphocytes; blood samples collected 10−11 am; processed immediately; Scored blind to exposure status; Comet assay, parameter: tail length, alkaline conditions (pH = 13), Singh et al. (1988) lysis 1 hr, 20 min electrophoresis, 100 cells/ subject, image analysis software; Cytokinesis-blocked MN test, Teixeira et al. (2004); culture incubation 72 hr; samples applied by smears to slides, stain 4% Giemsa; scored 1,000 binucleated cells/subject, scored	Selection & recruitment of exposed and referent not described. Participation rates not reported. Unexposed worked in administrative offices in hospitals in proximity to pathology labs	Exposed matched to unexposed by age, gender, lifestyle and smoking habits; unexposed worked in same area in administrative offices Demographic information provided	Analyses by one-way ANOVA and Student's t-test	Exposed n = 30; Referent n = 30	No obvious bias

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		blind by one reader, criteria Caria et al. (1995); SCE/ cell, 50 2nd division metaphases scored by one observer, Scored blind to exposure status					
Costa et al. (2011) (Portugal) Hospital pathology laboratories (*n* = 5) (prevalence)	Samples in breathing zone, NIOSH method #3500. Sampling duration, sample number was not given. 8-hr TWA calculated for each worker	Peripheral lymphocytes; blood samples collected 10–11 am; processed immediately; scored blind to exposure status; comet assay, parameter: tail length and % tail DNA; alkaline conditions, Singh et al. (1988) 100 cells/subject, image analysis software; Cytokinesis-blocked MN test Teixeira et al. (2004); culture incubation 72 hr; samples applied by smears to slides, stain 4% Giemsa; scored 1,000 binucleated cells/subject, scored	Selection & recruitment of exposed and referent not described. Participation rates not reported. Excluded exposed with <1 yr employment. Unexposed worked in administrative offices in hospitals in proximity to pathology labs.	Exposed matched to unexposed by age, gender, and smoking habits. Demographic information provided	Comet assay: normal distribution, analyses by one-way ANOVA and Student's *t*-test MN: not normal distribution, used nonparametric tests, Mann-Whitney U test and Kruskal-Wallis test	Exposed *n* = 48; Referent *n* = 50	No obvious bias.

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		blind by one reader, criteria Fenech (2007)					
Costa et al. (2013) (Portugal) Anatomy/ pathology lab workers	# samples and duration not reported. Air sampling in breathing zone. Calculated 8-hr TWA for each subject; NIOSH method # 3500	Peripheral blood samples collected between 10–11 am. Samples processed and assays conducted blinded. Cytokinesis-blocked MN test Teixeira et al. (2004). 1,000 cells analyzed/subject, MN per 1,000 binucleated cells, scored blindly by one reader, criteria Fenech (2007). SCE, scored 50 M2 metaphases/ subject by one reader T-Cell Receptor mutation assay in mononuclear leukocytes, # events in mutation cell window (CD3-CD4+ cells) divided by total	Included workers with at least 1-year employment in 4 hospital pathology anatomy labs; referent worked in administrative offices in same area & no occupational exposure history to formaldehyde	Similar in gender distribution, age, BMI, and smoking habit Demographic information provided	Difference in means, Student's *t*-test; tested for normal distribution multivariate analysis adjusted for age, gender, and smoking	Exposed *n* = 35; referent *n* = 35	No obvious bias

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		number of events for CD4+ cells					
Costa et al. (2015) (Portugal) Anatomy/ pathology laboratories	Samples in breathing zone for periods during formaldehyde-related tasks, NIOSH method #3500. Sampling duration, sample number was not given. 8-hr TWA calculated for each worker	Peripheral blood samples collected between 10–11 am. Samples processed and analyzed blinded. Chromosome aberrations (structural and numerical), duplicates cultured 51 hrs cited (Roma-Torres et al., 2006), 4% Giemsa stain; coded slides; scored 100 metaphases per person, 1,250× magnification; CTAs & CSAs according to Savage et al. (1976); gaps not included. Comet assay: alkaline conditions according to Singh et al. (1988); Scored blind 100 cells/donor from	Included workers with at least 1-yr employment in 4 hospital pathology anatomy labs; referent worked in administrative offices in same area & no occupational exposure history to formaldehyde; exclusions cancer/tumor history, radiation therapy or chemotherapy treatments, last year surgery with anesthesia and blood transfusions.	Similar distributions by exposure group for age, gender, and smoking. Evaluated possible confounding by other measures (diet) and found confounding by fruit consumption for frequency of multiaberrant cells and %tDNA.	Exposed compared to unexposed using Student's t test for ln % tDNA or Mann-Whitney U-test for CA measures; linear regression of ln %tDNA; negative binomial regression for untransformed total-CAs, CSAs, CTAs, gaps, aneuploidies, & aberrant cells; Poisson regression for untransformed multiaberrant cells. Models adjusted for age, gender and smoking plus actual confounders for specific parameters. Analyzed effect modification by genotype (homozygous variant plus heterozygous)	Exposed = 84; Unexposed = 87	No obvious bias

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		two gels; % DNA in comet tail.			compared to homozygous wildtype, genotype frequency compared by Pearson's chi-square test		
Costa et al. (2019) (Portugal) Anatomy/ pathology laboratories	Samples in breathing zone for periods during formaldehyde-related tasks and at other sites "considered relevant", NIOSH method #3500. Sampling duration and number were not given. 8-hr TWA calculated for each worker	Peripheral blood samples collected and processed and assays conducted blinded. Exfoliated cells were collected for each cheek separately. Cytokinesis-blocked MN test, Costa et al. (2008); culture incubation 72 hr; samples applied by smears to slides, stain 4% Giemsa; scored 1,000 binucleated cells/subject, scored blind by one reader, criteria defined by Fenech (2007) Buccal MN cytome assay. Scored blind by same reader, 2,000 differentiated cells scored for frequency of MN, nuclear buds	This study analyzed additional endpoints using blood and buccal cell samples collected in Costa et al. (2015). Selection & recruitment of exposed and referent not described. Participation rates not reported. Included workers with at least 1-year employment in 9 hospital pathology anatomy labs; referent worked in administrative offices in same area & no	Similar distributions by exposure group for age, gender, and smoking. Exposed smokers smoked less than unexposed smokers (11 versus 15 pack-yrs). Evaluated possible confounding by other measures (diet) and found confounding by fruit consumption for frequency of multiaberrant cells and %tDNA. The association of exposure with possible confounders was examined using linear regression. Dietary habits were	Sample size varied by endpoint because of "sample limitation and/or technical losses," although missingness likely not associated with exposure. Data were log transformed to approximate normal distribuion for TCR-Mf and Mann-Whitney U test applied to MN in lymphocytes and buccal cells and nuclear buds in buccal cells. Associations (mean ratio (MR), 95% CI) with SCE, MNB, BNbud and log TCR-Mf were assessed using Poison regression.	MNL Exposed = 84; Unexposed = 87 SCE/cell Exposed = 84; Unexposed = 87 MNB Exposed = 63; Unexposed = 69 BNbud Exposed = 63; Unexposed = 69 TCR-Mf Exposed = 61; Unexposed = 64	No obvious bias

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		and nucleoplasmic bridges according to Thomas et al. (2009); Tolbert et al. (1992). SCE/ cell, 50 2nd division metaphases scored by one observer, Scored blind to exposure status. T-Cell Receptor mutation assay in mononuclear leukocytes, flow cytometry, minimum of 2.5 × 10^5 lymphocyte-gated events were acquired, # events in mutation cell window (CD3-CD4+ cells) divided by total number of events for CD4+ cells	occupational exposure history to formaldehyde.	reported to be parameter-specific actual confounders for white blood cell counts.	Untransformed MNL also were modeled using negative binomial regression. Models adjusted for age, gender, smoking habits and dietary habits. Effect modification by genotype analyzed using Mann-Whitney U test for specific polymorphisms in CYP2E1, GSTM1, GSTT1, GSTP1, SRCC1, PARP1, MUTYH, RAD51 BRIP1 and FANCA.		
Fleig et al. (1982) (Germany) Formaldehyde manufacturing	Personal sampling, 8-hr shift, number of measurements or people with monitors not reported. Measurements were not	Chromosome aberrations, peripheral blood lymphocytes cultured 70–72 hrs, 10% Giemsa stain; coded slides.	Recruitment and selection of participants not described. Referent group from administrative or office staff at same	Referent matched to exposed by age and gender; stated smoking not associated with CA (data not reported)	Fisher-Yates exact test	Exposed *n* = 15, referent *n* = 15	Cell incubation period 72 hrs

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
	reported. Provided categories of maximum exposure as % of MAK value for 25%, 60%, and 100% of MAK for two periods (before and after 1971)	Presented aberrant cells/ individual both including gaps and excluding gaps	site with no formaldehyde exposure				
Gomaa et al. (2012) (Egypt) Pathology, histology and anatomy laboratories at a university	No formaldehyde measurements	Chromosome aberrations (structural and numerical), cited Verma (1998), peripheral blood lymphocytes cultured 72 hrs, 5% Giemsa stain; blinding not described; scored total CA and types, analyzed 50−100 metaphases per subject. Comet assay, alkaline conditions according to Singh et al. (1988); tail length & tail moment; blinding not described;	Recruitment and selection of participants not described. Referent group described to be unexposed	Age comparable between exposed and referent; data analysis by gender; no evaluation of smoking	Difference in mean values between exposed and referent, Student's *t*-test	Exposed *n* = 30, referent *n* = 15	Cell incubation period 72 hours; blinding not described; no evaluation of smoking

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		analyzed 50 cells per subject.					
Hayes et al. (1997) (USA) Panel study, 9 weeks embalming course Related to Suruda et al. (1993)	Personal sampling; cumulative exposure estimated using sampling data and time-activity data; continuous area samples at head height over embalming tables for short-term peak concentrations; monitored for other compounds: glutaraldehyde, methanol, isopropyl alcohol, and phenol	Blood samples collected in morning before 1st class and after 9 weeks; analysis blinded to exposure status; O^6-alkylguanine DNA alkyl-transferase activity in peripheral blood lymphocytes (according to Klein and Oesch, 1990), expressed as pmol AGT/mg protein (LOD 0.006 pmol AGT/ mg protein), blind to period of sample (before or after)	Recruited volunteers prior to beginning of course; reported loss to follow-up.	15 students had some prior embalming experience during lifetime; exposure to other chemicals below LOD or very low; confounding not likely	Change in individual; Individual data pre- and postcourse AGT activity in peripheral blood lymphocytes depicted in graphs by embalming experience during previous 90 days (yes/ no), ANOVA adjusting for age, sex, and smoking.	*N* = 29	No obvious bias, small sample size
He et al. (1998) (China) Prevalence Anatomy students	Breathing-zone samples during dissection; number, duration of sampling not described	Blood collection not described. Assays used whole blood. Cytokinesis-blocked MN assay, cultured 72 hr, cells processing (Fenech and Morley, 1985),	Recruitment and selection details not described. Demographic data comparing exposed and referent groups were not provided.	All nonsmokers, age and sex similar (data not reported)	Analytic method not described	Exposed *n* = 13 Referent *n* = 10 (# in table reported as 13)	Deficiencies and inconsistency in reporting, small sample numbers.

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		blinding not described (scored 1,000 cells per individual), CA analyzed 100 metaphases, modified fluorescence-plus-Giemsa stain; SCE analyzed 50 metaphases, Giemsa stain, Blinding not described					
Jakab et al. (2010) (Hungary) Hospital and university pathology department	Area samples, records of measurements within 1–3 yrs of study 8-hr TWA determined	Venous blood collection, timing not stated, peripheral blood lymphocytes HPRT gene mutations, unscheduled DNA synthesis, CA and SCE whole blood samples, cultures incubated 50 (CA) and 72 (SCE) hours; CA stain 5% Giemsa, SCE fluorescence plus Giemsa; analyses blinded, for CA scored 100 metaphases/ subject. Scored total CA and types, SCE and high	Recruitment and selection of participants not described. Participation rates not reported. Referent group from health-service staff in same hospitals	Provided data on demographic characteristics; Age comparable, Formaldehyde only group had higher proportion of smokers, more cigarettes/day and higher proportion drinkers. Solvents were ethyl alcohol, acetone, and xylene	Exposure groups compared, student's *t*-test SCE stratified by smoking, CA frequency analyses not stratified	HCHO alone *N* = 21; HCHO and solvents *N* = 16; Referent *N* = 37	Possible confounding by smoking on CA association not assessed. Direction: potential over-estimation

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		frequency SCE, total premature centromere division (PCD) and mitoses with >3 chromosomes with PCD					
Jiang et al. (2010) (China) Woodworkers (prevalence study)	Personal samples in breathing zone; 3–5 workers from each job title, 5 referent workers; 8 hr samples; calculated 8-hr TWA	Blood lymphocytes; blinded analysis; comet assay (DNA strand breaks), lymphocytes isolated within 2 hr after blood draw, alkaline conditions, (Singh et al., 1988); slides dessicated, shipped to Beijing, >100 cells/ subject, image analysis software. MN: cytokinesis-block micronucleus assay (chromosome damage), scoring criteria (Fenech et al., 2003) 1,000 binucleated lymphocytes/ subject	Selection & recruitment of exposed and referent not described. Participation rates not reported. 263 male workers all Han Chinese; 151 exposed from two plywood industries; 112 referents from a machine manufacturing plant in same town	Excluded subjects with recent exposure to known mutagenic agents (x-ray) chronic conditions (autoimmune disease), recent antibiotic use. Structured questionnaire collected info on smoking, alcohol, medical conditions, occupational history & house redecoration in last year. Evaluated mean age and frequency of smoking and alcohol by exposure level.	Ln-transformed Olive TM and CBMN frequency ANOVA differences by exposure group; *t*-test for differences in means. ANCOVA differences by years of exposure among exposed adjusted for age, formaldehyde concentration, smoking and alcohol.	Referent *N* = 112 Exposed *N* = 151	No obvious bias

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Kitaeva et al. (1996) (Russia) Translation Formaldehyde production and anatomy lab workers	Exposure definition by job task, no formaldehyde measurements	MN assay in buccal mucosal cells, blinding not described, cell collection using swab, smeared onto slides, stain Feulgen and light green, analyzed 2,000 cell/ subject. CA in peripheral blood (blood from finger), reported % metaphases with aberrations after 72-hrs culture; # metaphases at 72 hrs cultivation was low (148), observed in only 8 exposed workers	Recruitment and selection not described. Referent group not defined clearly.	Referents 10 years younger than exposed; Stated that age and smoking were not related to MN or CA frequency, gender not related among unexposed, Data not shown.	Analysis using Student method with Freeman-Tukey transformation and results were not clearly presented	Female Exposed *n* = 8 Female Referent *n* = 7; Students *n* = 12	Small numbers, reporting deficiencies for details of study design and results, difficult to evaluate
Kurttio et al. (1993) (Finland) Wood plywood/ veneer manufacture	No formaldehyde measurements; exposure defined by task; 5 out of 15 exposed, considered to be exposed to formaldehyde; referent selected from same town employed at municipal energy plant, a loading	Venous blood samples cultured all on same day; cultured for 48 hr according to (Jantunen et al., 1986); slides coded; analyzed 100 metaphases per subject	Selection of exposed and referents not described; referents were employed in other industries (potential for dis-similarities)	All male, matched on age, data analysis excluded one smoker	Structural aberrations, mean # per cell by exposure, Mann-Whitney U-test (2-tailed)	Exposed *n* = 15; Referent *n* = 15	5 out of 15 considered exposed to formaldehyde; no formaldehyde-specific data analysis **Not informative**

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
	company, or a health care center						
Ladeira et al. (2011) (Portugal) Histopathology labs in 6 hospitals	Personal air sampling, 6–8 hours, estimated 8-hr TWA (NIOSH method 2541) Ceiling values for each task	Cell collection between 10 am and noon. Samples coded and analyzed blinded. Peripheral blood lymphocytes, cytokinesis-block micronucleus cytome assay, fresh samples, cultured for 72 hr, applied to slides with cytocentrifuge, May-Grunwald-Giemsa, 1,000 binucleated cells scored/ subject by 2 readers; buccal mucosa cells, collection using endobrush, smeared onto slides, stain Feulgen, 2,000 cells scored/ subject, 2 readers	Recruitment and selection not described. Participation rates not reported. Excluded history of cancer, radio or chemotherapy, use of therapeutic drugs, exposure to diagnostic x-rays in the past 6 mos, intake of vitamins or other supplements like folic acid (no one was excluded)	Exposed were older, with lower proportion of drinkers and smokers	Comparisons by exposure group; binary logistic regression and Mann-Whitney test Stratified by categories of age, gender and smoking	Exposed *n* = 56, referent *n* = 85	No obvious bias
Lan et al. (2015) (China) Formaldehyde-melamine resin	Personal monitors for 3 d over entire shift within a 3-wk period.	Postshift and overnight peripheral blood samples. Metaphase spreads from colony forming	Analyzed aneuploidy among subset with scorable metaphases, high	Referents frequency-matched by age (5 yr) and gender	Analyzed using negative binomial regression controlling for age and gender. Also	Exposed *n* = 29; Referent *n* = 23	No obvious bias

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
production or use Bassig et al. (2016); related study related study Zhang et al. (2010)	Formaldehyde concentration: 8-hr TWA Exposed Median: 1.38 ppm (1.7 mg/m^3) 10th & 90th percentile: 0.78, 2.61 ppm 0.96, 3.2 mg/m^3) Referent 0.026 ppm (0.032 mg/m^3) 10th & 90th percentile: 0.015, 0.026 ppm (0.019, 0.032 mg/m^3) LOD: 0.012 ppm	unit granulocyte macrophage (CFU-GM) cultured for 14 d; chromosome-wide aneuploidy analysis using OctoChrome FISH; scored minimum 150 cells/subject; analysis blinded to exposure.	formaldehyde among exposed and existence of comparable referents. Participation rates exposed 92%, referent 95%. Referent from 3 workplaces in same geographic region as exposed, engaged in manufacturing with similar demographic and SES; excluded history of cancer, chemotherapy, and radiotherapy, previous occupations with exposure to benzene, butadiene, styrene, and/or ionizing radiation.	Personal sampling of volatile organic compounds; concentrations at background, urinary benzene at background and comparable between groups	evaluated potential confounding from current smoking and alcohol use, recent infections, current medication use, and body mass index (Supplemental tables in Supplemental tables in Lan et al., 2015)		
Lazutka et al. (1999) (Lithuania) Carpet and plastic manufacturing	Industrial hygiene area measurements reported by plant; carpet plant, formaldehyde	Peripheral blood samples; chromosome aberrations, cells cultured 72 hr, differential staining	Recruitment and selection not described. Participation rates not reported.; Source population	Nonexposed were "approximately" matched to exposed by age; males and females, smokers and	ANOVA including variable for exposure and age, no adjustment for smoking or gender; CA data	Carpet plant, exposed 38 male, 41 female; unexposed 64 male, 26 female	Cell incubation period 72 hours; unable to distinguish between

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Prevalence study	0.3−1.2 mg/m^3, styrene 0.13−1.4 mg/m^3, phenol 0.3 mg/m^3; plasticware plant, formaldehyde 0.5−0.9 mg/m^3, styrene 4.4−6.2 mg/m^3, phenol 0.5−0.75 mg/m^3	fluorescence−plus-Giemsa, CA scored on coded slides, >100 first mitotic division cells per subject.	for nonexposed referents not described	nonsmokers included; demographic information provided; unable to distinguish between formaldehyde and styrene	transformed using average square root transformation	Plastic plant, exposed 34 male, 63 female; unexposed 64 males, 26 females	formaldehyde and styrene effects Direction: potentially overestimated
Lin et al. (2013) (China) Woodworkers (prevalence study) 2009 (cross-shift) 2011	Prevalence: Area samples (2 badges in each of 5 workplaces with differing tasks), 8-hour samples on two days. Change over work-shift: badges in breathing zone of 2−4 representative workers conducting different job types (8-hour samples). Referent group exposed, mean 0.13 mg/m^3 (0.019−0.252)	Blood lymphocytes; blinded analysis; comet assay (DNA strand breaks), alkaline conditions (pH=13) (Olive and Banath, 2006), lysis 2-hr for *N* = 178 & over-night for *N* = 62, 50 lymphocytes/ sample, image analysis software; cytokinesis-block micronucleus assay, Fenech (1993) analyzed 1,000 binucleated cells/ subject, scoring criteria Fenech (1993), Fenech et al. (2003); Zhitkovich and	Selection & recruitment of exposed and referent not described. Participation rates not reported. Exposed and referent from same factory.	Excluded subjects with exposure to known mutagenic agents in previous 3 months (radiotherapy & chemotherapy). Structured questionnaire collected info on smoking, alcohol, medical conditions, occupational history, and house redecoration in last year.	Natural log-transformed olive TM. Prevalence: ANOVA differences by exposure group (control, low and high), adjusting for age, sex, smoking, alcohol, # work years) Regression for trend across exposure level adjusting same as above; Poisson regression for MN frequencies, linear regression for Ln(OTM) Across-shift: Paired Wilcoxon text (MN freq) or paired *t*-test (OTM or DPX); regression models	Referent *N* = 82 Low *N* = 58 High *N* = 38	Referent group with significant formaldehyde exposure, potential bias toward null.

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		Costa's KCl-SDS assay (DNA-protein crosslinks)			for trend with exposure levels		
Marcon et al. (2014) (Italy) Population living in proximity to chipboard plants	Modeled outdoor formaldehyde concentrations at residential address based on data from 62 monitoring sites in district; four 1-wk sampling periods (2 each in warm and cold seasons); calculated annual average concentration of formaldehyde and NO_2; estimated at each address using ordinary Kriging; formaldehyde 2.5 $\pm$ 0.3 $\mu g/m^3$, NO_2 16.0 $\pm$ 3.5 $\mu g/m^3$,	Epithelial mucosal cells using cytology brush; comet assay, alkaline conditions, 50 cells per subject; MN 2,000 cells per subject, according to Tolbert et al. (1991)	Random sample of participants in previous survey (93% of population in Viadana District) with children under 12 yrs, Italian primary language, and address information; invited stratified random sample in 3 strata of distance from wood factories (656 remaining in district since 2006 of 750), participation 63%, participation was not higher in residents closest to wood factories; higher proportion of nonparticipants were of foreign nationality and	No adjustment for indoor formaldehyde concentrations; co-exposure with NO_2	Linear regression for tail length, tail intensity, tail moment and binucleated cells; negative binomial regression for micronuclei and nuclear buds; models adjusted for children's sex, age, nationality, parents' education, parents' smoking, exposure to tobacco smoking at home, time with windows open, traffic near home, orthodontic appliance, condition of teeth, person who collected cell sample	*N* = 413; Analysis included only complete datasets for comet assay, *n* = 310 and MN *n* = 374	Potential exposure misclassification; no obvious bias

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
			had smoking parents				
Musak et al. (2013) (Slovakia) Prevalence study Pathologists	Air monitoring once per year (no details provided)	Chromosomal aberration, peripheral blood lymphocytes, blinded analysis, cultured 48 hr, 100 mitoses scored/ subject, 2 scorers	Recruitment and selection of participants not described. Participation rates not reported. Exposed and referent all employed in hospitals	Exposed and referent comparable for age, gender; % smokers slightly higher in exposed; analyses adjusted for age, gender, job type, and smoking	Adjusted odds ratios, Binary logistic regression controlling for age, gender, job type, and smoking	Exposed *N* = 105; Referent *N* = 250	No obvious bias
Orsiere et al. (2006) (France) Hospital pathology labs (prevalence)	Personal sampling near breathing zone; Short-term: 15 minutes, Long-term 8 hrs during typical work day.	Peripheral lymphocytes, blood samples taken preshift and postshift; processed within 6 hr, assays conducted blinded. Chemi-luminescence microplate assay; cytokinesis -blocked micronucleus assay Sari-Minodier et al. (2002); cultured 72 hr, smears on slides, stain 5% Giemsa, scoring criteria (Fenech, 2000)	Selection & recruitment of exposed and referent not described, however subgroups selected randomly. Exposed and referent worked in same institution.	Groups similar for gender, age, % smokers. No exposure to other genotoxic substances. Excluded history of radiotherapy or chemotherapy and use of therapeutic drugs that were known mutagens or reproductive toxicants	Differences by group analyzed using nonparametric Mann-Whitney U-test; median DNA repair across shift analyzed using Wilcoxon W-rank sum test. Analyzed binucleated micronucleated cell rate (BMCR), and MN measures using multivariate regression adjusting for smoking, drinking, age, and gender.	Exposed *n* = 59; referent *n* = 37; Subgroups Exposed *n* = 18; referent *n* = 18	No obvious bias.

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		; 1,000 binucleated cells/ subject; FISH with a pan-centromeric DNA probe, same operator scored exposed and referent blinded					
Pala et al. (2008) (Italy) Research institute lab (prevalence)	Personal samples, one 8-hr shift; 75% exposed to < 0.026 mg/m^3.	Peripheral blood samples collected at same time at end of day; processed within 20 hr; analysis blind to exposure. CA, harvested after 48 hr, 100 metaphases/ subject SCE, cultures harvested at 72 hr, analysis of 30 second-division cells/subject; MN: modified cytokinesis-blocked method, Fenech and Morley (1986); 72 hr incubation, stain 3% Giemsa, 2,000 cells/subject	Selection & recruitment of exposed and referent not described. Participation rates not reported.	Statistical models adjusted for gender, age, and smoking	Multivariate regression models adjusting for gender, age, and smoking; Poisson model for CA and MN, SCE log-normal random effects model, comparisons were low and high exposure groups, below and above 26 µg/m^3	*N* = 36	No obvious bias; only 9 exposed above 0.026 mg/m^3.
Peteffi et al. (2015) (Brazil) Furniture manufacturing	Monitoring in 7 sections in facility; referent monitoring in 5	Peripheral blood processed within 4 hr. comet assay, alkaline conditions according	46 workers in furniture manufacturing facility and	Exposed and referent had comparable distributions for	Nonparametric tests used because data were not normally distributed.	Exposed *n* = 46, referent *n* = 45	No obvious bias

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
	areas of university; breathing zone 8-hr samples collected on same day as biological samples. Urine samples collected at end of work day on 5th day of work; correlation of formaldehyde concentration in air with urinary formic acid concentration, r = 0.626, p <0.001	to Tice et al. (2000); silver nitrate staining according to Nadin et al. (2001); 100 cells/ person read by two independent observers (50 cells each). Blinding not stated, classified by visual scoring according to Anderson et al. (1994); 5 categories based on tail migration (0–IV) and frequency of damaged cells (sum of I–IV), damage index (Pitarque et al., 1999) Oral mucosa samples (scraped with endocervical brush), micronucleus test, DNA-specific Feulgen staining and counterstaining with Fast Green according to Tolbert et al. (1992); analyzed	unexposed group recruited from employees and students of local university with no history of occupational exposure to potentially genotoxic agents or substances metabolized to formic acid	age, smoking, and alcohol; differed by gender Exposed 56.5% male, referent 33.3% male; no association of any biomarkers with gender (data not shown)	Exposed and referent compared using Mann-Whitney test;		

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		2,000 cells/ person by 2 independent observers (1,000 ea)					
Santovito et al. (2014) (Italy) Hospital nurses	All exposed used protective equipment; no formaldehyde measurements, intensity and frequency likely highly variable	Peripheral blood samples, coded, processed within 2 hr after collection. Cultures incubated for 48 hr for CA and 72 hr for SCE; CA slides stained with 5% Giemsa, scored 200 metaphases per subject, gaps not scored as CA; SCE 50 metaphases scored per subject	20 female nurses from 2 analogous departments in 2 hospitals; 20 referents from administrative departments of same hospital; all nonsmokers and did not consume alcohol	Accounted for sex, age, smoking, and alcohol in design; referents from same hospitals Nurses exposed to other substances	Mean frequencies compared, Wilcoxon test; regression analysis, association of age and exposure duration on CA and SCE	Exposed *n* = 20; Referent *n* = 20	Potential for large degree of exposure misclassification and variation in intensity of exposure; bias toward null; small sample size
Santovito et al. (2011) (Italy) Pathology wards	Personal sampling near breathing zone, 8-hr duration	Venous blood sample collected at end of shift, samples coded and processed within 4 hr, same day concentration sampling conducted, cultured 48 hrs; CA 5% Giemsa stain; scored 100 metaphases/ subject	Recruitment and selection of participants not described; participation rates not reported.	All nonsmokers, nondrinkers, no drug use 1 year prior; no information on other exposures (acetone, ethyl alcohol, xylene)	Mean % of cells with aberrations and frequencies of aberrations per cell compared using Mann-Whitney U test, 2-tailed. Generalized linear models (Poisson distribution) adjusting for age, gender, polymorphisms, Cubic spline regression of mean % of cells with	Exposed *n* = 20; Referent *n* = 16	No obvious bias Small sample size

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
					aberrations and frequencies of aberrations per cell with number years exposed and age		
Schlink et al. (1999) (Germany) Anatomy students	Personal sampling near breathing zone once per week, sampling period not reported. formaldehyde exposed, Mean ± SD, 0.2 ± 0.05 mg/m^3, 0.14–0.3 mg/m^3	Blood samples collected before 1st class and after days 50 and 111; O^6-alkylguanine DNA alkyl-transferase activity in peripheral blood lymphocytes (modification of Klein and Oesch (1990), expressed as fmol MGMT/ 10^6 cells (LOD 1 fmol MGMT/ 10^6 cells), blind to period of sample (before or after)	Recruitment and participation of students were not described. 41 students from one university course, 16 students from a different university course, and 10 unexposed students	Considered effects of age, sex, smoking, and alcohol	MGMT activity change compared (U-test, paired data) within categories of sex, smoking, allergy, and alcohol; as well as between groups (Wilcoxon, Mann and Whitney U-test)	Exposed *N* = 41 Referent *N* = 10	No obvious bias, small sample size
Shaham et al. (1997) (Israel) anatomy/ pathology departments (prevalence) also reported in Shaham et al. (1996)	Personal and "field" samples, duration 15 min, multiple times during work day (# not reported).	Peripheral lymphocytes; DPX, K-SDS method; double blinded. SCE at 72 hrs, mean of 30 cells/ individual, blinding not described	Selection & recruitment of exposed and referent not described. Participation rates not reported. Referent group worked at same institution.	Exposed and referent matched by age (matching protocol not described). No exposure to other mutagens or substances known to cause DPX in either exposed or referent.	Analyses by ANOVA adjusting for smoking; difference in means, *t*-test; linear regression for DPX levels or means SCE per chromosome by years of exposure to formaldehyde	Exposed DPX: *N* = 12 SCE: *N* = 13 Referent DPX: *N* = 8 SCE: *N* = 20	Low sample numbers; no obvious bias.

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Shaham et al. (2002) (Israel) Hospital pathology labs	Personal and area samples, sampling at different points in work day, sampling duration averaged 15 min	SCE in peripheral lymphocytes, blood samples collected at same time in morning; blinding not described, stain fluorescence plus 5% Giemsa, scored 30–32 cells/subject	Recruitment and selection of participants not described. Referent group from administrative sections of same hospitals	Authors presented demographic data. Exposed were higher proportion female, European/ American, education >12 yr, and lower proportion smokers. No exposures to other chemicals linked to SCE. Confounding addressed in analysis	Mean # SCEs per chromosome and proportion of high frequency cells compared between exposed and referent. Difference between means assessed using ANOVA (unbalanced design) adjusting for age, gender, smoking, origin and education years	Exposed *n* = 90; Referent *n* = 52	No obvious bias
Shaham et al. (2003) (Israel) 14 hospital pathology departments (prevalence)	Personal and "field" samples, duration 15 min, multiple times during work day (# not reported).	Peripheral lymphocytes; DPX, same protocol as Shaham et al. (1997); SCE; pantropic p53	Selection & recruitment of exposed and referent not described. Exposed and referent worked in same institution.	Adjustment for age, sex, smoking, origin, and years of education in analysis. No exposure to other mutagens or substances known to cause DPX in either exposed or referent.	Analyses: comparisons of mean DPX adjusted for sex, smoking, age, origin, and years education. Comparison of mean DPX by low and high formaldehyde levels and by duration of exposure, Mann-Whitney test	Exposed *N* = 186; Referent *n* = 213	No obvious bias.
Souza and Devi (2014) (India) Prevalence study Anatomy Dept (embalming)	No formaldehyde measurements reported.	Total MN/1,000 cells peripheral lymphocytes. Assays conducted blinded. Cytokinesis -blocked	Recruitment and selection of participants not described.	Provided characteristics of exposure groups (see Table 1). All male, age	Frequency MN compared by exposure group using Student's *t*-test, and by	Exposed *N* = 30 Referent *N* = 30	No obvious bias

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		micronucleus assay Costa et al. (2008); stain 4% Giemsa, scoring criteria Fenech (2000), 1,000 binucleated cells/ subject. Frequency MN compared by exposure group using Student's *t*-test, and by duration of employment using Pearson's correlation.	Participation rates not reported.	comparable, higher prevalence smokers in exposed. Adjustment in analysis. Excluded frequent exposure to x-rays or other radiation, worked in paint or pesticide industries or history of chemotherapy.	duration of employment using Pearson's correlation. Exposure and smoking evaluated together using two-way ANOVA.		
Speit et al. (2007a) (Germany) Controlled human exposure study	Generation using para-formaldehyde; 10 consecutive days, 5 groups of 3−6 persons in chamber, 4 hr exposures, some exposures masked with ethyl acetate, 3 15-min exercise sessions during exposure; randomized order of concentration, double blinded	MN in buccal mucosal cells−1 wk before start, at time=0, after end of exposure, and 1, 2, and 3 wks after end of exposure; cells collected with metal spatula, smeared onto slides, blinded analysis at end of study by one person, stain DAPI/ propidium iodide, 2,000 cells/ subject	Excluded severe allergy, skin or airways disease, acute infection, current smoking or within last 3 yrs, contact lenses or glasses, > 50 g alcohol per day, present use of psychotropic agents, exposure to ionizing radiation, or cytostatic drugs during the last 6 mos	Within person comparison	Post exposure compared to preexposure using Wilcoxon ranked sum test	*N* = 21	No obvious bias.

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Suruda et al. (1993) (USA) Panel study, 85 d Embalming course	Personal sampling for 121 of 144 embalmings; cumulative exposure estimated using sampling data and time-activity data; Continuous area samples at head height over embalming tables for short-term peak concentrations; monitored for other compounds: glutaraldehyde, methanol, isopropyl alcohol, and phenol	Nasal mucosa cells, oral mucosa cells, blood samples collected in morning before 1st class and after 9 wks; processed on same day, analysis of slides blinded to exposure status; pre- and postslides from each subject stained at same time and read together by one reader, conducted a blinded 10% recount of slides; MN assay buccal and nasal cells Stich et al. (1982), collected with cytopathology brushes, slides prepared with cytocentrifuge, stain Feulgen/ Fast Green, 1,500 cell/ subject; MN lymphocytes Fenech and Morley (1985), stain Feulgen 2,000 cells/ subject;	Recruited volunteers prior to beginning of course; reported loss to follow-up. Excluded one student with many embalmings in previous 90 d, & one students who chewed tobacco during study	21 students had some prior embalming experience during lifetime; exposure to other chemicals below LOD or very low, confounding not likely	Change in individual; difference in mean pre- and postexposure, matched Student's *t*-test (SCE) or Wilcoxon sign-rank test (micronuclei); Change with cumulative exposure spearman's rank correlation coefficient & linear regression (if residuals were normally distributed)	*N* = 29	No obvious bias

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		SCE 50 s division metaphases scored/ subject					
Suskov and Sazonova (1982) (USSR) Phenol-formaldehyde resin production	Area samples, # and duration not reported	Cytogenetic analysis in peripheral lymphocytes; Chromosomal aberrations, blinding not described, Buckton and Evans cytogenetic method, 1973	Recruitment and selection not described.	Average age in exposed 39.1 yr, referent 34 yr. Matched for gender, smoking, alcohol, and medication (data not shown)	Compared chromosome aberration frequency by exposure group, chi-square	Exposed *n* = 31; Referent *n* = 74	Brief report, minimal detail of methods
Thomson et al. (1984) (Great Britain) Pathology lab	Sampling in breathing zone; 26 samples taken for the duration of the task involving formaldehyde exposure, over 1–3 mos, sample duration not reported, calculated TWA Measured peaks in breathing zone on one day for different tasks	CA frequency, stain fluorescence plus Giemsa technique Perry and Wolff (1974), cells harvested 48 hr, slides coded and scored 100 1st division metaphases/ subject; SCE frequency, cells harvested 72 hr, 50 cells/subject; blinding not reported	All exposed worked in same laboratory; characteristics of referent not provided.	Obtained smoking histories	Data analysis not described	Exposed *n* = 6; referent *n* = 5	Reporting of study methods and group characteristics not adequate; low sample numbers
Titenko-Holland et al. (1996) Same subject as	See Suruda et al. (1993) Calculated 2 exposure periods:	Buccal cells, Scored previously unstained and unanalyzed slides. New method: FISH with a centromeric	Subjects with missing MN data were compared to those with complete data by Student's *t*-test;	Change in individual. Exposure to other chemicals below LOD or very low,	Change in total MN, MN- and MN+ frequency (per 1000 cells) and change in mean MN. Excluded subjects with <500	Complete MN data from buccal mucosa, *n* = 19	No obvious bias

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Suruda et al. (1993) (USA) Panel study, 90 d Embalming course	(1) Lagged 7−10 d before last sampling to account for lag in development of MN (2) 90-d cumulative	probe—differentiates between clastogenic vs aneuploidogenic mechanism (total MN, MN- and MN+); <1,500 cells scored for 14 of 35 subjects; scored pre- and postexposure slides at same time, blinded. Frequency calculated by dividing # cells with MN by total # cells counted, multiplying by 1,000. 78% of preexposure slides and 76% of postexposure slides were scorable; 10% of slides were rescored	comparable for age, smoking, and mean exposure	confounding not likely	epithelial cells available for analysis. Difference scores evaluated using Wilcoxon sign-rank test. Association with both formaldehyde exposure metrics via Spearman non-parametric correlation coefficient, two-sided *p*-values	Complete MN data from nasal mucosa, *n* = 13	
Vasudeva and Anand (1996) (India) Medical student lab	<1 ppm, no data reported to support assertion	Peripheral blood lymphocytes, frequency of aberrant metaphases; cell culture 72 hr, Giemsa staining, blinding not reported	Recruitment and selection of participants not described. No demographic information provided.	Stated that participants had received no or insignificant radiation treatments (no data reported); exposed and referents matched by age, no other potential confounders evaluated	Data analysis not described	Exposed *n* = 30; referent *n* = 30	Reporting of methods, design and results not adequate to evaluate; cell incubation 72 hr

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Viegas et al. (2010) (Portugal) Formaldehyde & resin production, pathology/ anatomy lab workers Also discussed in Viegas et al. (2013)	Personal air sampling, (*N* = 2 in factory, *N* = 29 in labs) 6–8 hrs, estimated 8-hr TWA (NIOSH method 2541). Ceiling values for each task	Buccal mucosa Peripheral blood lymphocytes, sample collection between 10 am & noon. Blinded coding and analysis, buccal cell MN cell collection using endobrush, smeared onto slides, Feulgen stain, 2,000 cells scored/ subject by 4 observers, scoring criteria Tolbert et al. (1992), peripheral lymphocytes, samples processed within 6 hr, cultured for 72 hr, applied to slides with cytocentrifuge, stain May-Grunwald-Giemsa, 1,000 binucleated cells scored/ subject	Recruitment and selection not described. Participation rates not reported.	Presented comparisons for gender, age, and smoking. Difference by gender (higher prevalence males in exposed); genotoxic endpoints were not associated with smoking or gender, and only slightly with age	Correlation evaluated using Pearson or Spearman correlation test depending on distribution	Exposed, Produc-tion *n* = 30, Lab workers *n* = 50, Referent *n* = 85	No obvious bias
Wang et al. (2019) (Shanghai, China) Chemical production	Routine formaldehyde monitoring by factory with sampling site selection using China national standard for	CBMN according to Fenech (2000), Fenech (1993). Blinded analysis. Venous peripheral blood cultured for 44 hr, Cytochalasin-B	Recruitment and selection of participants not described; participation rates not reported. 100 male workers exposed to	Mean age and frequency of smoking and alcohol use were slightly higher in exposed. Work duration was higher in exposed.	MN frequency compared using Poisson regression and frequency ratio (FR) as effect estimate. Exposure was analyzed with quartiles for	Exposed n = 100 Unexposed n = 100	No obvious bias

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
	hazardous substances air sampling in the workplace. Cumulative dose determined for each worker (C × T). C = geometric mean of concentration for a year at a sampling site, T = years. Serum formaldehyde-albumin adducts (FA-HSA) quantified in fasting venous peripheral blood. Geometric mean range (mg/m^3): Exposed: 0.06–0.25 Unexposed: 0.01	added to cultures, cells harvested 28 hours later, air dried slides stained with Giemsa, MN dectected at 400× with confirmation at 1,000×. 1,000 binucleated cells scored/ subject	formaldehyde > 1 year through 4 work processes (i.e., production examination, glue spraying, coating and workplace inspection). Demographic information, smoking and alcohol, medical and occupational history (job types and # years) collected by questionnaire. Unexposed group (n = 100 males) from the logistics workshop in same factory age matched (likely frequency matched since rates were different)	Age, smoking status and alcohol use were adjusted in statistical models.	cumulatiave dose and FA-HSA concentration. Cumulative dose (mg/m^3): 0.01–0.06 0.06–0.125 0.125–0.9 0.9–3.75		
Yager et al. (1986) (USA) Anatomy course, 10 wks	Area samples randomly distributed (*N* = 13, 1−4/ wk); breathing zone samples on 30	Whole blood cultures; stain fluorescence plus Giemsa technique, Mean SCEs per cell in peripheral lymphocytes; before	Recruitment and selection not described.	All nonsmokers, 7 female	Paired *t*-test of before and after samples	*N* = 8	No obvious bias

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
	individuals at 15 tables (*N* = 35, 2–8/ week), mean sampling duration 18 min	and after samples coded and randomized together for analysis, scored 80 cells/subject					
Vargová et al. (1992) (Czechoslovakia) Woodworking	8-hr sampling duration in breathing zone	CA frequency, peripheral lymphocytes, Giemsa staining, cells harvested 48 hr, 100 cells/ subject. Blinding not described. CA frequency in both exposed and referent was higher than range considered normal	Recruitment and selection of participants not described; participation rates not reported.	Referents were matched to exposed (did not report what matching parameters were), no info on subject characteristics was reported Authors stated questionnaire data suggested that factors such as smoking and alcohol were different between exposed and referent; analyses were not adjusted.	Exposed and referent compared using student's *t*-test and arcsin-sqrt transformation test	Exposed *n* = 20 (or 25?); Referent *n* = 19	Reporting of study methods and group characteristics not adequate; # exposed in text did not match # exposed in table II in the paper. Lack of adjustment for confounding, bias toward null
Ye et al. (2005) (China, 1992) Formaldehyde exposure in factory or indoor	Sampling according to NIOSH method; Referent *n* = 6; Waiters *n* = 18; Workers *n* = 36	MN in nasal mucosa, cell collection using swab, cells smeared onto slide, stain Wright's, scoring criteria Sarto et al. (1987), per 3,000	Recruitment and selection not described. Included: nonsmokers, no medicines for 3 wks prior and during study, no x-	Waiters and workers older than referent, % male 52% in referent, 25% in workers, 61% in wait staff; all Han Chinese; no adjustment for age	Analysis using one-way ANOVA and tested for multiple comparisons. Data presented in figures and values estimated from graph by EPA.	Workers *n* = 18; waiters *n* = 16; referent *n* = 23	Possible bias away from null; expect higher frequency of MN in older individuals. Small sample numbers.

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
air from building materials		cells, blinding not stated, results reexamined by another trained staff. SCE in peripheral lymphocytes, time of sample not stated; stain Giemsa solution, analysis blinded, 30 M_2 lymphocytes analyzed/subject.	ray history for 6 months prior, no drug use; comparison groups were from different sources: industrial exposed, wait staff (indoor air exposed), and unexposed student volunteers	or gender in analyses.			
Ying et al. (1997); Ying et al. (1999) (China) Panel study, 8-wk class Anatomy students	NIOSH (1977) method; 3-hr TWA and peaks; sample duration, number and frequency not described	Nasal mucosa cells, oral mucosa cells, blood samples collected before 1st class and after last class; analysis of slides by one blinded observer with reexamination by another, nasal and buccal cells collected with swab, smeared onto slides, MN Nasal and Buccal cells, Wright's stain, scored 4,000 cells/ subject; MN blood lymphocytes, stain 4% Giemsa, scored mean of 2,870−3,167 cells/ subject; MN scoring criteria Sarto et al.	Included nonsmokers, students living in dorms, disease-free & no medications prior 3 wks, no x-ray history prior 6 mos	Mean age 18.8 ± 1.0 yr, all Han nationality, all lived in dorms, all nonsmokers	Change in individual over time; paired *t*-tests	*N* = 25	No obvious bias, small sample size

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		(1987), SCE and LTR (Zhao et al., 1994): 30 M_2 lymphocytes per subject analyzed blind to exposure					
Zendehdel et al. (2017) I(ran) Melamine dinnerware manufacturing Related publication: Zendehdel et al. (2018)	Personal air sampling, NIOSH method 3500, whole shift for each worker. Median time weighted average in three workshops, 0.086 mg/m^3; range, 0.02–0.22 mg/m^3; authors state that 2/3 of sample were exposed to < 0.1 mg/m^3	Comet assay, alkaline conditions, according to Tice et al. (2000) Blood samples collected same day as air sampling; blinding not described; minimum of 50 randomly selected cells per sample; tail moment and Olive moment	Workers in 3 melamine dinnerware manufacturing workshops (n=49) and referents matched by age and sex (n=34) who worked in food industries, # smokers higher in referent (26% versus 16%), >90% male. Recruitment and participation were not described.	Data in Table 1 of paper supported comparability of age, sex, and # smokers in exposed and referent groups.	Normal distribution assessed using Kolmogorov-Smirnov test. Difference in mean tested using Student *t*-test or Mann-Whitney test	Exposed N = 49; Referent N = 34	No obvious bias blinding not described;
Zhang et al. (2010) (China) Formaldehyde-melamine resin production or use Related publications: Bassig et al.	Personal sampling for full shift (>240 min) on 3 working days over 3 wks. Exposed: at least 2 samples per individual; Referent: Sampling in subgroup on 1 d.	Postshift and overnight peripheral blood samples; analysis blinded to exposure. Metaphase spreads from cultured colony forming unit granulocyte macrophage (CFU-	Participation rates exposed 92%, referent 95%. Referent from 3 workplaces in same geographic region as exposed, engaged in manufacturing with similar	Referents frequency-matched by age (5 yr) and gender	Analyzed using negative binomial regression (exposed compared to unexposed) controlling for age, gender, and smoking	High *N* = 10 Low *N* = 12	Small sample numbers, no obvious bias

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
(2016); Gentry et al. (2013); (Mundt et al., 2017) Reanalyses	Evaluated for other known or suspected leukemogens (benzene, phenol, chlorinated solvents), found none. Analysis blinded.	GM); identified loss of chromosome 7 and gain of chromosome 8 using FISH	demographic and SES; excluded history of cancer, chemotherapy, and radiotherapy; previous occupations with exposure to benzene, butadiene, styrene and/or ionizing radiation.		Mundt et al. (2017) presented individual data in graphs for chromosome 7 and chromosome 8, noting smoking status and whether 150 or more cells were evaluated. Gentry et al. reported that < 150 cells per individual were analyzed for several subjects. Not expected to be different between exposed and unexposed, impact likely to increase variability and attenuate association		

Summary Table by Genotoxicity Endpoint

A text summary of the available genotoxicity data that emphasizes genotoxicity studies incorporating inhalation formaldehyde exposure and related experiments (i.e., given the known toxicokinetics of inhaled formaldehyde) is provided in Section 1.2.5 (Evidence on Mode of Action for Upper Respiratory Tract Cancers). Table A-27 below provides a summary of the most relevant data organized by genotoxicity endpoint, as compared to the organization by test system in the previous sections. In addition, when possible, this table separates the summary into investigations of respiratory- versus nonrespiratory-related tissues or systems. Thus, observations of genotoxicity in the upper respiratory tract (URT) and in peripheral blood lymphocytes (PBLs) following inhalation exposure or in related in vitro systems are presented in Table A-27 in order of their importance and relevance to cancer risk beginning with gene mutations, DPXs and DDCs, DNA adducts, CAs, MN, DNA strand breaks, SCE, and other effects. Overall, the evidence supports the conclusion that formaldehyde is genotoxic. Particular weight is placed on the following observations:

1) Consistent observations of mutations in exposed rodents and various in vitro systems;

2) Observations of CAs, MNs, and SSBs in exposed humans across a range of studies, occupations, and exposure scenarios, with supporting, similar findings in exposed rodents and in vitro systems; and

3) Consistent observations of DPX detected in multiple experimental systems, showing a concentration-dependent increase, and concordance of DPX distribution with sites of tumors in the nose.

Table A-27. Genotoxicity summary table

Genotoxicity endpoint(s)	Experimental system	Genotoxicity evidence (in descending relevance)	Other relevant information or limitations	Endpoint summaries	Endpoint conclusion
Gene Mutations	Respiratory tract tissues or in vitro systems	+(1/2) In vivo, rodent (inhalation); + 1/1 chronic; 0/1 subchronic studies + (5/5) In vitro, human cell lines, acute studies +(8/10) In vitro, rodent cell lines, acute studies +(13/17) Nonmammalian systems	In vivo rodent studies analyzed SCCs from a chronic study and non-neoplastic nasal mucosa from a subchronic study at 18.45 mg/m^3 All in vitro studies assume MeOH co-exposure; cellular sources both POE and systemic sites Negative in vitro rodent data for HPRT; + results include colony formation and mutation frequency	Mutations induced by formaldehyde across a range of in vitro systems. Mutations observed in SSC in nasal tissues of exposed rodents at 18.45mg/m^3 in one chronic inhalation study.	Observation of gene mutations in nasal SSC in one chronic-duration rodent study (which only tested high formaldehyde levels), with confirmatory evidence from in vitro test systems across several species. No mutations in subchronic-duration rodent study. No studies of exposed humans or primates.
	Other tissues	+(1/2) in vivo, rodent (inhalation); dominant lethal studies +(1/2) in vivo, rodent (i.p.); dominant lethal mutation studies	Formalin inhalation exposure at 200 mg/m^3 prevents interpretation; another inhalation study at 1.5 mg/m^3 was equivocal i.p. exposure with MeOH co-exposure caused + DLM in rats (0.125 mg/kg), but not in mice (20 mg/kg) at much higher levels	Results are interpreted as equivocal; the available studies do not provide evidence of mutations in other tissues	
Chromosomal aberrations (CA)	Respiratory tract tissues or in vitro systems	+(1/1) in vivo, rodent (inhalation): short term study +(4/4) In vitro, human cells/cell lines, acute studies +(5/6) In vitro, rodent cell lines, acute studies	In vivo rat study at 18.45 mg/m^3 with 4-wk exposure In vitro studies assume co-exposure to MeOH; cell sources both POE and systemic sites 1 equivocal CA study in a rodent cell line	CAs were observed in the only in vivo rodent study, which is supported by positive results in human and rodent cells in vitro.	Evidence from exposed humans across several different occupations is consistent with the induction of CAs. These results are supported by observations of CAs in the only available in vivo rodent study (4 wks at high levels), which was consistent with findings from multiple in vitro studies of human and rodent cells lines

Genotoxicity endpoint(s)	Experimental system	Genotoxicity evidence (in descending relevance)	Other relevant information or limitations	Endpoint summaries	Endpoint conclusion
	Other tissues	+(11/16) in vivo, human (inhalation): PBLs +(1/5) in vivo, rodent (inhalation): short term studies +(2/2) in vivo, rodent (gavage, p.o.): acute studies +(1/4) in vivo, rodent (i.p.): acute or short term studies	In humans, half + CAs were observed in pathologists and half among industrial workers; often, these studies involved relatively higher formaldehyde exposure levels (e.g., average >0.2 mg/m^3) and longer employment duration (e.g., average >10 yr) The only positive rodent inhalation study involved MeOH co-exposure*; 4 studies used PFA Oral exposure in rats and mice involved MeOH co-exposure, although 1 study indicated it takes >10× MeOH to cause a similar level of CAs The + i.p. study was in rat bone marrow cells after 4-wk exposure; − studies were acute, mice studies	Most of the human studies interpreted with higher confidence observed increased CA in PBLs; Lower exposure levels may explain null findings. Rodent results are interpreted as equivocal. The rodent studies do not provide evidence that CAs are induced in other tissues; however, the data suggest the possibility that rats might be more sensitive and that exposure duration is important.	
Micronuclei (MN)	Respiratory tract tissues or in vitro systems	+(11/13) in vivo, human (inhalation); +(0/1) in vivo, rodent (inhalation); short term study +(5/5) In vitro, human cell line; acute study +(4/4) in vitro, rodent cell lines; acute studies +(1/3) nonmammalian studies	MN reported in buccal and nasal cells, occupational (average >0.5 mg/m^3), anatomy or embalming courses (average >0.5 mg/m^3 with intermittent peaks). No increase after 5−10 d in 2 controlled human exposure studies, In vivo rat study at 18.45 mg/m^3 for 4 wk (in BAL) MN observed in primary human blood cultures, and in 3 in vitro rodent studies with no MeOH co-exposure; remaining cell studies assume MeOH; cellular sources both POE and systemic sites	Consistently increased frequency of MN or related endpoint in buccal and/ or nasal cells of exposed individuals Consistent evidence of MN across a range of in vitro mammalian cells, but not in a short term rodent inhalation study.	Available evidence suggests increased MN levels associated with cumulative exposure; the pattern of chromosomal loss (monocentromeric and multi-centromeric micronuclei) was consistent with aneuploidy in exposed individuals

Genotoxicity endpoint(s)	Experimental system	Genotoxicity evidence (in descending relevance)	Other relevant information or limitations	Endpoint summaries	Endpoint conclusion
	Other tissues	+(11/16) in vivo, human (inhalation) PBLs, +(1/2) in vivo, rodent (inhalation); short-term studies +(1/5) in vivo, rodent (i.p., i.v., p.o. or gavage); acute studies	MN reported in PBLs of workers from plywood and formaldehyde production industry, and pathology, anatomy, and mortuary lab students, at exposure concentrations of 0.1−0.5 mg /m^3. Null results in studies with low sensitivity. No increase after 5 days in controlled human exposure study. Prevalence increases with longer exposure duration. In rodents, MN were in bone marrow erythrocytes at 12.8 mg/m^3 with 10-wk exposure, but not in peripheral blood at 18.45 mg/m^3 with 4-wk exposure. The + non-inhalation study was an oral rat study of gastric epithelial cells; all − studies were in mice	Most of a large set of studies that measured MN in PBLs reported increased levels among exposed participants working in diverse exposure settings and in several countries. The two rodent inhalation studies suggest the possibility that MN induction may require longer exposure duration, but results were mixed; data suggest the possibility that rats might be more sensitive.	
Aneuploidy	Respiratory tract tissues or in vitro systems	+(1/3) In vitro, human cell lines; short-term studies +(1/3) in vitro, rodent cell lines; short-term studies	All negative in vitro studies have co-exposure with MeOH	Inconsistent results from in vitro human or rodent cell lines; Methanol co-exposure is likely to influence the aneuploidy in cultured cells	Chromosome aneuploidies are consistent with study findings of CA and mono-centromeric and multicentromeric micronuclei in PBLs of exposed humans
	Other tissues	+(3/4) in vivo, human (inhalation) +(1/3) in vitro, rodent cell lines +(1/3) in vitro, human cell lines	An occupational study in humans reported monosomy 7 and trisomy 8 in cultured CFU-GM colony cells from peripheral blood. Analysis of same cohort with bigger sample size detected aneuploidy in several chromosomes. Two in vitro studies each from rodent and human cell lines used MeOH-free HCHO, one positive study in human cells has co-exposure with MeOH.	Significant increase in chromosome aneuploidy in cultured CFU-GM colony cells among subset of highly exposed workers compared to matched controls	

Genotoxicity endpoint(s)	Experimental system	Genotoxicity evidence (in descending relevance)	Other relevant information or limitations	Endpoint summaries	Endpoint conclusion
DNA adducts	Respiratory tissues or in vitro systems*	+(2/2) in monkeys (inhalation) hm-DNA adducts +(3/4) in rats (inhalation) hm-DNA adducts +(2/2) in vitro human cell lines, hm-DNA adducts +(1/1) in vitro rodent cell lines, hm-DNA adducts +(10/10) in cell-free systems, hm-DNA adducts	No in vivo studies in humans showing hm-DNA adducts with a direct exposure to formaldehyde. Detectable hm-DNA adducts in all nasal passages, but not in lungs of rats. High endogenous hm-DNA adduct levels rats and monkeys, but monkeys > rats	All tissues in nasal passages demonstrated hm-DNA adducts except lung tissue of rodents. Endogenous levels of hm-DNA adducts are very high in both rats and monkeys compared to exogenous hm-DNA adducts. Monkeys have much higher endogenous hm-DNA adduct levels compared to rats.	Formaldehyde readily forms hm-DNA adducts in tissues at POE. However, available evidence does not show their formation in distal tissues.
	Other tissues	+(1/1) in vivo, human, M_1G adduct +(0/2) in vivo, monkeys (inhalation), acute studies +(0/2) in vivo, rodent (inhalation), acute studies	One study reported M_1G adducts in peripheral blood of pathologists, uncertainties with regard to site of DNA interactions. hm-DNA adducts were not found in distal tissues of exposed monkeys or rodents	Absence of hm-DNA adducts in distal tissues suggest lack of formaldehyde transport to distal sites. Limited evidence of formaldehyde-induced oxidative DNA damage.	
DDC	Respiratory tissues or in vitro systems*	+(1/1) in vivo, rat (inhalation), acute study +(3/3) in vitro, cell-free systems	Only one in vivo study reports DDC. But DDC are unstable and could be generated as an artifact.	Limited evidence of DDC formation by formaldehyde in vivo.	Limited evidence that formaldehyde inhalation results in DDC although artifacts were not ruled out.
	Other tissues	+(0/1) in vivo monkey (inhalation) short-term study +(0/1) in vivo rat (inhalation) short-term study	DDC were not detectable in distal tissues.	DDC have not been detected in distal tissues	
DNA-Protein Crosslinks	Respiratory tissues or in vitro systems*	+(1/1) in vivo, monkeys (inhalation), acute study +(7/11) in vivo, rodents (inhalation), acute studies +(30/30), in vitro, human cell lines, acute studies +(21/21) in vitro, rodent cell lines, acute studies +(3/3) nonmammalian systems +(4/4) cell-free systems	Concentration-dependent increase in DPX in rodents (0.37–12.1 mg/m^3) and monkeys (0.86–7.37 mg/m^3); DPX demonstrated in nasal mucosa of rats but absent from olfactory mucosa and lung; a negative study in BAL cells used formalin vapors	Consistent evidence of DPX across multiple test systems (two species in vivo, different cell lines, nonmammalian and cell-free test systems)	Anatomical distribution of DPX in rats corresponds to sites of tumor incidence, cell proliferation, and cytotoxicity in the nose. However, no mechanism is identified for DPX formation in PBLs of occupationally exposed individuals.

Genotoxicity endpoint(s)	Experimental system	Genotoxicity evidence (in descending relevance)	Other relevant information or limitations	Endpoint summaries	Endpoint conclusion
	Other tissues	+(2/3) in vivo, human (inhalation) PBLs +(4/8) in vivo, rodent (inhalation)	Occupational settings, one null study of plywood workers had low sensitivity (referent group had high exposure), no difference in prevalence by exposure group, but increase in DPX was observed over 8-hr shift. Positive rodent studies have co-exposure with MeOH.	In vivo human studies show exposure duration-dependent increase in DPX in PBLs, but animal in vivo studies are confounded by MeOH coexposure.	
DNA strand breaks	Respiratory tissues or in vitro systems*	+(1/1) in vivo, rodent (inhalation), short-term study +(10/12) in vitro, human cells, acute studies +(3/7), in vitro, rodent cells/cell lines, acute studies +(4/4) nonmammalian systems	Only one in vivo study and several cell culture studies reports SSB formation, but most of these studies have co-exposure with MeOH. Human cells were more sensitive to SSB formation by HCHO exposure (0.005−0.8 mM) Excision-repair deficient yeasts were more sensitive compared to repair-proficient strains.	Single strand breaks in rat study were positively associated with concentration.	Some evidence for SSB with dose-response in respiratory tissues from an inhalation study in rats, and consistent evidence in PBLs from several studies of human exposure and from rodent studies
	Other tissues	+(8/9) in vivo, human (inhalation) PBLs, +(3/4) in vivo, rodent (inhalation), short-term studies	Exposure settings were occupational with means > 0.2 mg/m^3, 1 controlled human exposure study (4-hr duration). Categorical analysis by one study showed exposure-response trend beginning at 2nd quintile (mean 0.14 mg/m^3) Positive rodent in vivo studies have co-exposure with MeOH.	Consistent evidence of SSB formation in both human and rodent in vivo studies	
Sister chromatid exchange (SCE)	Respiratory tissues or in vitro systems*	+(6/6) in vitro, human cells/cell lines, short-term studies +(13/14) in vitro hamster cell lines, short-term studies	Positive studies included mostly co-exposure with MeOH, but several studies in both human and animal cell lines, which used methanol-free formaldehyde, were also positive.	Consistent evidence of SCE formation from in vitro human and rodent cell lines	No in vivo studies in animals, and less consistent results in exposed humans
	Other tissues	+(8/16) in vivo human (inhalation) PBLs +(0/3) in vivo, rat (inhalation) short-term studies	Several studies of occupational exposure showed increased SCE levels. Although MeOH-free or MeOH-co-exposed rat studies were negative, male rats received MeOH-free formaldehyde were positive in bone marrow cells.	Evidence that SCE is induced in some exposed human populations, although the results across studies are not consistent	

Genotoxicity endpoint(s)	Experimental system	Genotoxicity evidence (in descending relevance)	Other relevant information or limitations	Endpoint summaries	Endpoint conclusion
Other effects (cell transformation; DNA repair inhibition; unscheduled DNA synthesis; gene conversion, crossing over and translocation)	Respiratory tissues or in vitro systems*	+(4/7) in vitro, human primary cells/cell lines, (2/5 UDS) and (2/2 DNA repair inhibition, short-term studies +(4/5) in vitro, rodent cell lines, short-term studies (1/1 UDS; 3/4 cell transformation) +(8/8) nonmammalian system; [(1/1) DNA repair inhibition; +(2/2) gene conversion; +(3/3) genetic crossing over/recombination; +(2/2) heritable translocation]	Although most of the in vitro and nonmammalian studies were positive for other genotoxic effects, these studies had co-exposure with MeOH.	Available evidence suggests a variety of other genotoxic endpoints induced by formaldehyde exposure, which may play a supplemental role in overall genotoxicity.	Many of the other genotoxic endpoints support the overall genotoxicity and mutagenicity of formaldehyde across multiple experimental systems.
	Other tissues	+(1/2) *in vivo* human (inhalation)	Change in O6-alkylguanine DNA alkyl-transferase activity in PBLs before and after 2- to 3-month exposure in embalming or anatomy labs	Evidence is inadequate to conclude effect on DNA repair inhibition	

A.5. SUPPORT FOR HAZARD ASSESSMENTS OF SPECIFIC HEALTH EFFECTS

Supporting information is described for sensory irritation (Section A.5.2); pulmonary function (Section A.5.3); respiratory and immune-mediated conditions, including allergies and asthma (Section A.5.4); respiratory tract pathology (Section A.5.5); mechanistic evidence for potential noncancer respiratory health effects (Section A.5.6); respiratory tract, lymphohematopoietic, and other cancers (Section A.5.9); nervous system effects (Section A.5.7); and developmental and reproductive toxicity (Section A.5.8). The supporting information includes documentation of literature search methods and specific considerations for evaluating individual studies to determine their usefulness for assessing the health hazards of formaldehyde inhalation. General approaches used in the identification and evaluation of individual studies are summarized in Section A.5.1, with additional details outlined under each of the evaluated hazards. Because formaldehyde exposure-related issues were a significant concern in this assessment, a separate description of the considerations for judging exposure assessments in observational epidemiology studies is included (Section A.5.1, Exposure Assessments for Observational Epidemiology Studies), and all experimental studies considered for use in hazard identification, including controlled exposure studies in both humans and animals, were separately evaluated to assess the quality of the inhalation exposure protocols (Section A.5.1, Exposure Quality Evaluation: Animal Toxicology and Controlled Human Exposure Studies). Quantitative methods (e.g., benchmark dose modeling) applied to health effect studies considered for use in deriving reference values or cancer risk estimates are presented in Appendix B.

A.5.1. General Approaches to Identifying and Evaluating Individual Studies

Literature Search Methods

Literature search strategies involved keyword-based queries of the following literature databases: PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) and Web of Science (https://apps.webofknowledge.com/), with many of the health effect-specific searches including additional queries of Toxline (https://toxnet.nlm.nih.gov/newtoxnet/toxline.htm) and/or DART (https://toxnet.nlm.nih.gov/newtoxnet/dart.htm). Updates to the computerized searches were performed annually (i.e., either September or October) through 2016, after which point a separate systematic evidence map was developed to capture newer literature. For searches through 2016, the computerized search results were augmented by secondary search approaches, including curation of reference lists in published reviews and other national or international health assessments of formaldehyde. Studies were screened for relevance to this toxicological review based on inclusion and exclusion criteria organized according to PECOO category (Population, Exposure, Comparison, Outcome, and Other) considerations. This screening was performed using

title and abstract information or hand curation of the full text articles (when screening decisions could not be made based on the abstract) in Endnote libraries, and all of the screening decisions are documented in the formaldehyde page of the U.S. EPA Health Effects and Research Online (HERO) database (https://hero.epa.gov/hero/). Studies identified as relevant to assessing the health hazards of formaldehyde inhalation based on the criteria for the individual health effect searches were evaluated for use in the assessment.

Evaluation of Individual Observational Epidemiology Studies

Epidemiology studies were evaluated for several aspects of bias and sensitivity that could influence interpretation of study results, including population selection, exposure (measurement and levels/range), outcome ascertainment, consideration of confounding, and analytic approach. The potential for selection bias, information bias (relating to exposure and to outcome), and confounding were evaluated, and an overall confidence classification was developed for each study (or for a specific analysis within a study) (see Table A-28). The confidence classifications are "high," medium," "low," and "not informative." In some cases, sufficient information was available to allow characterization of the potential direction of bias (i.e., a low confidence study with a likely over-estimation of the effect estimate). For each study, the evaluations are recorded for each category, and the confidence classifications for specific endpoints are depicted in a diagram with text summarizing key limitations.

Table A-28. Approach to evaluating observational epidemiology studies for hazard identification

High Confidence *(highly informative)*	• No concern for bias, *AND* • Study design is highly informative for the outcome in question, *AND* • Analyses were appropriate and robust
Medium Confidence *(informative, with limitations[2])*	• Bias may be present but not expected to have strongly influenced the effect estimates, *AND* • Study design and analyses were informative for the outcome in question
Low Confidence *(minimally informative)*	• Methodological limitations are significant, but the study results might still be of limited use (e.g., as support for observations from other studies; to identify potential data gaps) *AND/OR* • Bias is apparent or other study aspects reduced sensitivity
Not Informative *(excluded as critically deficient)*	• Major concerns exist regarding methodological limitations that increased risk of bias, *OR* • Description of methods and/ or results were not adequate to enable a complete evaluation

Confidence classifications were developed for each study by integrating the judgements for each category of bias and sensitivity: population selection, information bias, confounding, analysis, and other (sensitivity). Some considerations included in the expert evaluations included:

Population Selection: Recruitment, selection into study, and participation independent of exposure status and reported in sufficient detail to understand how subjects were identified and selected.
Information Bias: Validated instrument for data collection described or citation provided. Outcome ascertainment conducted without knowledge of exposure status. Timing of exposure assessment appropriate for observation of outcomes. Information provided on the distribution and range of exposure with adequate contrast between high and low exposure.
Potential for confounding: Important potential confounders addressed in study design or analysis. Potential confounding by relevant co-exposures addressed.
Analysis: Appropriateness of analytic approach given design and data collected; consideration of alternate explanations for findings; presentation of quantitative results.
Other considerations not otherwise evaluated: Sensitivity of study (exposure levels, exposure contrast, duration of follow-up, sensitivity of outcome ascertainment).

Controlled human exposure studies were evaluated for important attributes of experimental studies including randomization of exposure assignments, blinding of subjects and investigators, and inclusion of a clean air control exposure and other aspects of the exposure protocol. The evaluation of few individuals ($n \leq 10$) resulted in reduced confidence. Several studies did not describe the measures used to control bias, resulting in a lower level of confidence in these study results. However, some of these studies evaluated multiple dose levels, an important strength for the hazard assessment. Therefore, these studies were included with *medium* confidence when reporting detail was the only identified limitation.

Evaluation of Individual Experimental Animal Studies

Experimental animal studies were evaluated and assigned the following confidence ratings: *High*, *Medium*, or *Low Confidence*, or "*Not Informative,*" based on expert judgement of each study's experimental details related to predefined criteria within five study feature categories: exposure quality, test subjects, study design, endpoint evaluation, and data considerations and statistical analysis. These evaluations were conducted for each independent "experiment" (i.e., a cohort of exposed animals assessed for an endpoint or set or related endpoints). Considerations for several of the criteria can differ depending on what endpoint is being evaluated; thus, a study with multiple experiments may be evaluated several times, with differing end results. The criteria were assessed independent of the direction, magnitude, or statistical significance of the experimental results, and they inform the reliability of the study findings regarding whether these findings are likely to be caused by formaldehyde exposure alone. Notably, the criteria are evaluated with regard to the study's ability to inform the health outcome being evaluated, which may differ from the author's intended purpose. *High* to *Low Confidence* studies represent the most to least useful experiments for the endpoint(s) in question, respectively, for use in hazard identification (see Table A-29).

Table A-29. Approach to evaluating experimental animal studies for hazard identification

High Confidence *(highly informative)*	• No notable methodological limitations, *AND* • Experimental design is highly informative[a] for the outcome in question
Medium Confidence *(informative, with limitations[b])*	• Minor concern regarding methodological limitations, *AND/ OR* • Experimental design is informative for the outcome in question
Low Confidence *(minimally informative)*	• Methodological limitations are apparent and significant, but the study results might still be of limited use (e.g., as support for observations from other studies; to identify potential data gaps) *AND/ OR* • Experimental design is minimally informative for the outcome in question
Not Informative *(excluded as critically deficient)*	• Major concerns exist regarding methodological limitations, which are expected to be a driver of study results, *OR* • Experimental design is noninformative for the outcome in question

[a]Considerations for whether the experimental design is informative include the value (e.g., sensitivity; specificity) of the methodological approaches for informing the outcome in question, based on known or expected biology and common practice. These considerations include, but are not limited to: appropriateness and sufficiency of exposure timing and/or duration to allow for the outcome to be affected; sensitivity and specificity of the endpoint assays regarding their ability to detect subtle changes in the outcome; and how well the tested animals (e.g., based on what is known about insensitive species, strains, or sexes) are able to reveal the outcome (note: the human relevance of the response is not considered at this point).

[b]As the expectation is that experimental studies should attempt to control all variables, any study limitation capable of influencing the data was considered to have negatively affected the reliability of the results. Studies were categorized as Medium Confidence if they had specific issues which introduce a limited amount of uncertainty regarding the interpretation of the results as solely attributable to formaldehyde inhalation exposure.

Documentation of the expert judgement evaluations within each of the study feature categories generally emphasized the identification of observed or potential limitations that might decrease confidence in the results, with less emphasis on documenting study-specific details that were interpreted as sufficient for the criteria preferences. These category-specific judgements were then used to assign the overall determinations of confidence (with the criteria most pertinent to determining confidence clearly identified). In general terms (specifics are provided for each hazard outcome evaluation in Appendix A.5.1–A.5.9), the five experimental feature categories evaluated in experimental animal studies involved the following considerations:

Exposure Quality: Given the importance of the inhalation exposure paradigms used across the available experimental animal studies, detailed evaluations of exposure quality were separately performed for each study (see below, Exposure Quality Evaluation: Animal Toxicology and Controlled Human Exposure Studies).

Test Animals: The species, sex, strain, and age are considered appropriate and sensitive for testing the endpoint(s); sample size provides reasonable power to assess the endpoint(s); overt systemic toxicity is absent or not expected at the tested concentrations, or it is

appropriately accounted for. Groups appear to be adequately matched at the onset of the experiment.

Study Design: The study design is appropriate and informative for evaluating the endpoint(s), including a sufficient exposure duration and/or appropriate timing of endpoint evaluations to allow for sensitive detection of the effect(s) of interest, and a lack of additional variables introduced over the course of the study that would be expected to modify the endpoint(s).

Endpoint Evaluation: The protocols used to assess the endpoint(s) are sensitive (able to detect subtle changes in the health outcome of interest), complete (include the appropriate protocol controls), discriminating (specific for the health outcome in question), and biologically sound (note: this applies to evaluations of novel or unproven methods regarding their ability to detect the changes in the endpoints of interest). The potential for experimenter bias is minimized.

Data Considerations and Statistical Analysis: Data for all endpoints evaluated in the study are presented with sufficient detail (e.g., variability is included) and in the preferred form (e.g., arbitrary cut-offs were not applied to continuous data). Statistical methods and the group comparisons analyzed appear to be completely reported, appropriate, and discerning (note: when inappropriate statistical methods appear to have been used, EPA sometimes performed additional comparisons).

Evaluation of Individual Mechanistic Studies

In general, studies relevant to mechanistic interpretations informing hazard identification were not individually evaluated. Rather, the body of evidentiary support (or lack thereof) for specific, influential mechanistic events (e.g., those known to be associated with the health outcome of interest; those previously implicated in authoritative reviews as relevant to interpreting formaldehyde exposure-induced health effects) were considered in totality, with judgements based on overarching interpretations across sets of related studies.

However, in several instances where a reasonable number of studies were available, but the mechanistic interpretations were not well-established, the individual mechanistic studies were systematically evaluated. For evaluations of individual mechanistic studies in experimental animal studies (i.e., mechanistic studies related to respiratory effects; mechanistic studies related to nervous system effects) the same general features evaluated for more apical measures of toxicity were considered (i.e., evaluations of exposure quality and study design were emphasized), although the specific criteria were simplified to accommodate the increased heterogeneity of the available mechanistic studies, as compared to more traditional apical measures of toxicity. Similarly, study evaluations of individual human studies (i.e., mechanistic studies related to respiratory effects; human studies of genotoxicity endpoints) emphasized consideration of exposure assessment, study design, outcome ascertainment, and comparison groups for potential sources of bias and their potential impact.

Evaluation of Exposure in Individual Studies

Exposure Assessments for Observational Epidemiology Studies

All residential or school-based studies with measures of formaldehyde exposure were included in the hazard identification evaluation. Because the database of studies with direct measurements is relatively large, residential studies with indirect measures of formaldehyde exposure (e.g., based on age of building or presence of plywood) were not included. Most of the included studies attempted to estimate average formaldehyde levels using area samples placed in one or more locations, with measurement periods ranging from 30 minutes to 2 weeks. A few studies included more than one sampling period (i.e., sampling on multiple days in different seasons over the course of a year). Studies in adults and in children indicate that area-based (e.g., residential or school) samples are highly correlated with personal samples (Lazenby et al., 2012; Gustafson et al., 2005); therefore, the use of measures based on residential (e.g., bedroom) samples rather than personal samples was not considered to be a limitation when evaluating a study. Formaldehyde concentrations have been found to be uniform throughout the home in both standing housing stock and mobile homes (Clarisse et al., 2003; Quackenboss et al., 1989b; Sexton et al., 1989; Stock, 1987; Dally et al., 1981). Therefore, associations have generally been analyzed using household average concentrations.

The validity of the measurement of average formaldehyde concentration was assessed by reviewing the description of sampling methods provided in each study. Indoor average formaldehyde measurements may be influenced by humidity and temperature, season, number of rooms sampled, sample placement, ventilation, and specific sources of formaldehyde in the building (Dannemiller et al., 2013; Salthammer et al., 2010). Longer sampling periods (e.g., 1- to 2-weeks duration) were considered to be reflective of usual average exposure levels experienced by occupants. Studies have shown that formaldehyde levels levels remain relatively stable over a series of days or weeks (Gustafson et al., 2005; Hodgson et al., 2000; Quackenboss et al., 1989b; Stock, 1987), although concentrations are also correlated with season, which reflects the influence of temperature and humidity (Dannemiller et al., 2013; Jarnstrom et al., 2006; Clarisse et al., 2003). Within-person variability increases with shorter sampling durations (Gustafson et al., 2005). However, indoor formaldehyde concentrations have not been found to be associated with indoor combustion sources, such as active smoking or ETS exposure, and cooking with gas stoves or wood burning (Mullen et al., 2015; Dannemiller et al., 2013; Gustafson et al., 2005; Clarisse et al., 2003; Stock, 1987; Hanrahan et al., 1984; Dally et al., 1981). Study evaluations looked for information regarding factors that influence formaldehyde levels as well as quality control measures and/or citations for exposure protocols. The following characteristics were examined to assess the potential bias and informativeness of the exposure measures in the observation epidemiology studies of formaldehyde in residences and schools:

- Duration of exposure measurement period and number of sampling occasions

- Consideration of temperature, relative humidity, and a discussion of quality control

- For shorter exposure periods (< 1 day), details regarding measurement protocol (e.g., shutting windows) and consideration of influence of sources of exposure (e.g., smoking or appliances)

- Limit of detection (LOD) and percent <LOD

- Ability to examine variability in risk in relation to variability in exposures above 0.010 mg/m^3; the ability is based on the distribution of exposure, specifically the upper portion of the distribution (e.g., 75th percentile) or the range of exposure encompassed within the study population (e.g., the degree of contrast between "high" and "low" exposure). A study that does not include values above 0.010 mg/m^3 would not be able to detect variation in risk in relation to variation in exposure typically seen in indoor settings.[13]

- Information about the distribution of formaldehyde encompassed by the study (at least one descriptive statistic, preferably denoting a point on the upper part of the distribution such as the 75th or 95th percentile). EPA's analysis is based on a comparison across studies of results, taking into account exposure levels; thus, it is not possible to interpret the results of a study that does not indicate the exposure levels that are being studied.

There was also variation in the exposure measurements used within occupational settings. For hazard identification, an accurate characterization of "high" versus "low" exposure or "exposed" versus "nonexposed" may be able to provide a sufficient contrast to examine associations, even if there is considerable heterogeneity within the high-exposure group. Exposure assessments in occupational studies involved one or more area samples in specific task areas, personal samples, or a combination of both. Sampling periods ranged from less than 1 hour to an entire work shift over 1 or more days. Concentrations were reported as an average over all samples for a particular location or as a time-weighted average (TWA) over the sampling period. Generally, a TWA concentration from a full shift measurement using personal sampling was considered a more precise estimate of exposure. Some occupational groups (i.e., embalmers, pathologists, wood or garment industry) were considered to be highly exposed to formaldehyde and were included despite the absense of sampling data.

<u>Exposure Quality Evaluation: Animal Toxicology and Controlled Human Exposure Studies</u>

Inhalation toxicity studies are particularly challenging because of the inherent complexity of generating and characterizing consistent chamber atmospheres. Poor study design, human error, and problems with mechanical and electronic equipment can impair an inhalation exposure and undermine the validity of a study. In experimental studies, there is an expectation that test subjects in an inhalation chamber study will be exposed solely to a well-characterized test article under conditions that are carefully regulated, frequently measured, and clearly reported. When a

[13]Note that this criterion applies specifically to formaldehyde and the conditions examined in this review; the relevant exposure range for other exposures or conditions could be very different.

chamber study is conducted under Good Laboratory Practice (GLP) standards, there is typically greater confidence that all aspects of that study were properly performed and documented.

Inhalation studies were evaluated by scientists familiar with inhalation chamber operations for seven key elements of exposure quality:

1) **Generation Method:** The equipment and method used to generate a chamber atmosphere should be clearly described. If methods from another publication are cited, the methods in the secondary article were evaluated (if accessible).

2) **Test Article Characterization:** The test article is the substance or mixture of substances to which humans or animals are exposed. Any substances used to generate the test article should be well characterized. For example, formaldehyde gas can be produced by heating paraformaldehyde, formalin, UFFI insulation, or Delrin plastic. The test article description should ideally include its physical nature (solid, liquid, gas, etc.), purity, CAS registry number (if known), and physicochemical properties (including isomerization and radiolabeling). Because inhaled methanol (but not formaldehyde) is systemically distributed and can cause neurological and developmental effects, a methanol control group is desirable for studies of commercial formalin. Only 2 of 84 studies known or believed to have tested commercial formalin included methanol controls.

3) **Analytical Method:** The method used to measure test atmospheres should be clearly described and suitable for the test chemical. There are specific methods (e.g., direct sampling, adsorptive, or chemical reactive methods, and subsequent analytical characterization such as HPLC, gas chromatography, etc.) and nonspecific methods such as gravimetric filter analysis. In addition, a real-time monitoring device (e.g., an aerosol photometer for aerosols or a total hydrocarbon analyzer for gases or vapors) may be used to monitor the stability of chamber atmospheres.

4) **Analytical Concentrations:** Every chamber study should report three concentrations, which are listed in the order of their usefulness:

- The **analytical concentration** is the analytically measured concentration of a substance to which test subjects are exposed in their breathing zone. Because analytical concentrations are recorded throughout the course of a chamber study, they can reveal generation problems, fluctuations, analytical problems, and missed exposures. If analytical concentrations are not reported for a study considered for use in quantitative analyses, an effort should be made to acquire them from the study authors, as analytical concentrations are preferred when deriving an RfC. The use of target or nominal concentrations to derive an RfC should be cited as a study limitation, although nominal concentrations are considered accurate for gases (but not vapors).

- The **nominal concentration** is the mass of generated test article divided by the total volume of air passed through the chamber. Nominal and analytical concentrations for gases are usually quite close. Conversely, the nominal concentration for a vapor or aerosol is typically greater than the analytical concentration (sometimes orders of magnitude greater) due to test chemical clumping, precipitation, and/or deposition on chamber walls and plumbing.

- The **target concentration** is the concentration the study director hopes to achieve in a chamber study (e.g., 1, 3, and 10 mg/m^3). Because a target concentration is a goal—not a measurement—one should not assume that test subjects were actually exposed at the precise target concentrations.

- Some fluctuation in analytical chamber concentration is expected, but concentrations should deviate from the mean chamber concentration by no more than ±10% for gases or vapors or ±20% for liquid or solid aerosols (GD 39, GD 39, OECD, 2009). Excessive atmosphere fluctuation is evidence of a test article generation problem.

5) **Particle Size Characteristics:** Particle median diameter, density, and distribution (geometric standard deviation or σg) should be characterized whenever test subjects may be exposed to an aerosol or to a vapor that may condense into inhalable aerosol particles. Particle sizing is not necessary when testing a gas. The mass median aerodynamic diameter (MMAD) is often calculated, but metrics such as physical diameter, median particle number, or surface area may also be evaluated as the most relevant metric.

6) **Chamber Type:** Inhalation chambers are either dynamic or static. Dynamic chambers, which include nose-only, head-only, and whole-body chambers, have a constant flow of filtered air and consistent test article concentrations, but static chambers do not. EPA and OECD inhalation test guidelines indicate use of a dynamic chamber. Static chamber studies are not preferred for longer term hazard identification or exposure response analyses in particular, as they can lead to a harmful buildup of by-products (e.g., CO_2). Consideration should also be given to whether the test article is best delivered by whole-body or nose-only chambers. Animals exposed to an aerosol in a whole-body chamber may receive a significant oral exposure due to preening of particles deposited on their fur. To prevent this, nose-only chambers are recommended when testing aerosols and vapors that may precipitate into particles.

7) **Controls:** A concurrent negative (air) control group should be used in inhalation toxicity studies. The test chamber, itself, is considered an experimental variable that should be controlled.

Inhalation study deficiencies are shaded in Table A-30 for easy recognition. A study's exposure quality may be upgraded if a study author provides key missing data. Each study was subjectively ranked as having **Robust**, **Adequate**, or **Poor** exposure characterization based upon the number and severity of deficiencies it has:

- **Robust Exposure Characterization:** There are no notable uncertainties or limitations regarding exposure methodology.

- **Adequate Exposure Characterization:** There are minor uncertainties or limitations regarding exposure methodology.

- **Poor:** There are serious uncertainties or limitations regarding exposure methodology.

Table A-30. Inhalation exposure quality: formaldehyde (Note: exposure deficiencies are shaded)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Robust Exposure Characterization: there are no notable uncertainties or limitations regarding exposure methodology						
Adams et al. (1987) **Mouse**	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Ahmed et al. (2007) **Mouse**	Paraformaldehyde	NR	HPLC	Reported	NA	Dynamic whole-body
Albert et al. (1982) **Rat** *See* Sellakumar et al. (1985)	Paraformaldehyde	—	—	—	—	—
Andersen et al. (2010) **Rat**	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Appelman et al. (1988) **Rat**	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic whole-body
Babiuk et al. (1985) **Rat**	Paraformaldehyde (and 7 other aldehydes)	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Bach et al. (1990) **Human** [Exposure parameters are inferred from coauthor using same climate chamber in Anderson and Mølhave, Andersen and Molhave (1983)]	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic "climate chamber"
Barrow (1983) **Mouse and Rat**	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry and colorimetric method	Reported	NA	Dynamic head-only
Battelle (1981) See (Kerns et al., 1983)	Paraformaldehyde	—	—	—	—	—

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Berglund and Nordin (1992) **Human**	Freshly prepared formalin from paraformaldehyde (no methanol)	Evaporation	IR spectrophotometry; sodium bisulfite method; acetyl acetone method	Reported	NA	Dynamic olfactomer
Berglund et al. (2012) **Human**	Freshly prepared formalin from paraformaldehyde (no methanol)	Evaporation	IR spectrophotometry; acetyl acetone method	Reported	NA	Dynamic olfactometer
Casanova et al. (1994) **Rat**	Paraformaldehyde, [^{14}C]-paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Cassee et al. (1996b); Cassee et al. (1996a) **Rat**	Freshly prepared formalin from paraformaldehyde (no methanol) and/or acetaldehyde, acrolein	Evaporation	Formaldehyde analyzer	Reported	NA	Dynamic nose-only
Cassee and Feron (1994) **Rat**	Freshly prepared formalin from paraformaldehyde (no methanol). Exposures were to PFA only, ozone only, or to both chemicals	Evaporation	IR spectrophotometry	Reported	NA	Dynamic nose-only
Chang et al. (1981) **Rat and mouse**	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry and colorimetric method	Reported	NA	Dynamic head-only
Chang et al. (1983) **Rat and mouse**	Paraformaldehyde and [^{14}C]-paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body and head-only
1982) *See* Kerns et al. (1983)	Paraformaldehyde	—	—	—	NA	—
Coon et al. (1970) **Rat, guinea pig, rabbit, dog, monkey**	Freshly prepared formalin (paraformaldehyde added to hot distilled water; 1.35% solution)	Spray nozzle and evaporation of solution	IR analyzer equipped with a catalytic oxidizer	Reported	NA	Dynamic whole-body
Dalbey (1982) **Hamster**	Paraformaldehyde	Thermal depolymerization	Colorimetric analysis	Within 5% of target	NA	Dynamic whole-body

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Dallas et al. (1989) **Rat**	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Day et al. (1984) **Human**	UFFI off-gas products	Broken-up UFFI foam was dampened with water, then gases collected in 4500 L polyethylene balloons.	Chromotropic acid	Reported	NA	Dynamic whole-body
Dean et al. (1984) **Mouse**	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Dinsdale et al. (1993) **Rat** *Experiment 2* *(See also Experiment 1-* ***Inadequate****)*	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Feron et al. (1988) **Rat**	Paraformaldehyde	Thermal depolymerization	Colorimetric	Reported	NA	Dynamic whole-body
Fujimaki et al. (2004b) **Mouse**	Paraformaldehyde	NR	HPLC	Reported	NA	Dynamic whole-body
Green et al. (1987) **Human**	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic whole-body
Green et al. (1989) **Human**	Paraformaldehyde	Thermal depolymerization	Colorimetric monitor	Reported	NA	Dynamic whole-body
Groten et al. (1997) **Rat**	Paraformaldehyde alone or in combination with dichloromethane, aspirin, di(2-ethylhexyl)-phthalalate, cadmium chloride, stannous chloride, butyl hydroxyanisol, loperamide, and spermine	Vaporization of freshly made formalin	Colorometric method	Reported (sampled in the animals' breathing zone)	NA	Dynamic whole-body

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Hayashi et al. (2004) **Mouse**	Paraformaldehyde	Thermal depolymerization	HPLC	Reported	NA	Dynamic whole-body
Holmstrom et al. (1989b) **Rat**	Paraformaldehyde with and without wood dust	Thermal depolymerization	Formaldehyde meter	Reported	NA	Dynamic whole-body
Jakab (1992) **Mouse**	Paraformaldehyde; exposure was to formaldehyde gas with or without carbon black aerosol	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Kamata et al. (1997) **Rat**	Formalin with 10% methanol A methanol control group was used	Sprayed into a bottle heated to 70°C	Acetylacetone	Reported for formaldehyde and methanol	NA	Dynamic nose-only
Kerns et al. (1983); 1982); Battelle (1981); Swenberg et al. (1980a) **Rat and mouse**	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Kulle et al. (1987) **Human**	Paraformaldehyde (reference provided)	Thermal depolymerization	Toxic gas monitor, chromotropic acid	Reported	NA	Dynamic whole-body
Kulle (1993) **Human**	Paraformaldehyde (reference provided)	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic whole-body
Kuper et al. (2011) **Rat**	Probably freshly prepared formalin (10.21% formaldehyde)	NR	IR spectrophotometry	Reported	NA	Dynamic whole-body
Larsen et al. (2013) **Mouse**	Polyacetal (a formaldehyde polymer) in permeation tubes	Permeation tube in a Kin-Tek gas standard generator	HPLC	Reported	NA	Dynamic head-only
Martin (1990) **Rat**	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic whole-body

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Monteiro-Riviere and Popp (1986) **Rat**	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Monticello et al. (1991) **Rat**	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Monticello et al. (1996) **Rat**	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Monticello and Morgan (1997) **Rat** Based on Monticello et al. (1996)	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Morgan et al. (1986a) **Rat**	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	±5% of nominal	NA	Dynamic head-only
Morgan et al. (1986c) **Rat**	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Mueller et al. (2012) **Human**	Paraformaldehyde	Thermal depolymerization	Formaldehyde monitor, HPLC	Reported	NA	Dynamic whole-body
Mueller et al. (2013) **Human**	Paraformaldehyde	Thermal depolymerization	Formaldehyde monitor HPLC	Reported	NA	Dynamic whole-body
Ozen et al. (2002) **Rat**	Paraformaldehyde	Thermal depolymerization	Gas chromatography and formaldehyde monitor	Reported	NA	Dynamic whole-body
Reuzel et al. (1990) **Rat**	Paraformaldehyde	Thermal depolymerization	Formaldehyde monitor	Reported	NA	Dynamic whole-body
Riedel et al. (1996) **Guinea pig**	Formaldehyde gas	Pressurized bottles	Photometric	Reported (in animals' breathing zone)	NA	Dynamic whole-body
Roemer et al. (1993) **Rat**	Paraformaldehyde	Thermal depolymerization	IR spectrophometry	Within 10% of nominal	NA	Dynamic head-only

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Rusch et al. (1983) **Rat, monkey, hamster**	Freshly prepared formalin (unstabilized 5% solution with 0.03% methanol)	Air was bubbled through formalin	Chromotropic acid	Reported	NA	Dynamic whole-body
Saldiva et al. (1985) **Rat**	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic whole-body
Sauder et al. (1986) **Human**	Paraformaldehyde (reference provided)	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic whole-body
Sauder et al. (1987) **Human**	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic whole-body
Sellakumar et al. (1985) and Albert et al. (1982) **Rat**	Paraformaldehyde; exposure to formaldehyde and/or HCl. Co-exposure to formaldehyde and HCl forms bis(chloromethyl)-ether (BCME), a carcinogenic reaction product.	A slurry of PFA in paraffin oil (kerosene) was generated by thermal depolymerization. HCl was from a compressed gas tank.	PFA: Chromotropic acid HCl: titration with NaOH BCME: gas chromatography/mass spectrometry	Reported [NOTE: HCl is a powerful catalyst for the polymerization of formaldehyde into oligomers (Bevington and Norrish, 2012). Unlike formaldehyde gas, oligomer particles may be respirable]	NA	Dynamic whole-body
Sheppard et al. (1984) **Human**	Freshly prepared formalin from paraformaldehyde (methanol-free)	Air was bubbled through formalin	IR spectrophotometry	Reported	NA	Respiratory valve mouthpiece
Songur et al. (2003) **Rat**	Paraformaldehyde	Thermal depolymerization	Formaldehyde monitor	Reported	NA	Dynamic whole-body
Songur et al. (2008) **Rat**	Paraformaldehyde	Thermal depolymerization	Formaldehyde monitor	Reported	NA	Dynamic whole-body
Sorg et al. (2001a) **Rat** [Cited exposure parameters from Sorg et al. (1998)]	Paraformaldehyde	Thermal depolymerization	Photoacoustic multi-gas monitor	Reported	NA	Dynamic whole-body

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Swenberg et al. (1980b) *See Kerns et al. (1983))*	Paraformaldehyde	—	—	—	NA	—
Swiecichowski et al. (1993) **Guinea pig**	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic whole-body
Tobe et al. (1985b) [Study report] **Rat**	Formalin (w/10% methanol) A methanol control group was used	Sprayed into a heated glass bath	Acetylacetone	Reported for formaldehyde and methanol	NA	Dynamic whole-body
Tsukahara et al. (2006) **Mouse**	Paraformaldehyde	NR	HPLC	Reported	NA	Dynamic whole-body
Usanmaz et al. (2002) **Mouse**	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic Not described
Vosoughi et al. (2013) **Mouse**	Paraformaldehyde	Thermal depolymerization	Photoionization detector	Reported	NA	Dynamic
Wood and Coleman (1995) **Mouse**	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Reported. Animals were able to stop irritating formaldehyde exposure	NA	Dynamic whole-body
Woutersen et al. (1987) **Rat**	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic whole-body
Woutersen et al. (1989) **Rat**	Paraformaldehyde	Thermal depolymerization	Colorimetric	Reported	NA	Dynamic whole-body
Zeller et al. (2011) **Human**	Paraformaldehyde	Thermal depolymerization	HPLC and formaldehyde monitor	Reported	NA	Dynamic whole body
Zitting et al. (1982) **Rat**	Polyacetal plastic (Delrin®)	Oxidative thermodegradation (250°C) to formaldehyde, formic acid, and acrolein	Visible absorption spectrometry (NIOSH, 1972)	Reported	NA	Dynamic whole-body

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Zwart et al. (1988) **Rat**	Paraformaldehyde	Thermal depolymerization (Woutersen et al., 1987)	Colorimetric	Reported	NA	Dynamic whole-body (reference provided)
Adequate Exposure Characterization: there are minor uncertainties or limitations regarding exposure methodology.						
Andersen (1979); also described in Andersen and Mølhave (1983) **Human**	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Within 20% of target	NA	Dynamic whole-body
Andersen et al. (2008) **Rat**	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry, HPLC	Reported (≈30% variation in atmospheres)	NA	Dynamic whole-body
Andersen and Molhave (1983) [book chapter] **Human**	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Within 20% of target	NA	Dynamic "climate chamber"
Apfelbach and Weiler (1991) **Rat**	Paraformaldehyde	Thermal depolymerization	HPLC	NR	NA	NR Exposures in plexiglas holding cages
Aslan et al. (2006) **Rat**	Paraformaldehyde	Thermal depolymerization	Formaldehyde monitor	NR "Desired concentrations were prepared"	NA	Dynamic whole-body
Bender et al. (1983) **Human**	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	NR14	NA	Dynamic smog chamber with 7 sets of ports
Boja et al. (1985) **Rat**	Paraformaldehyde	Thermal depolymerization	Gas chromatography	NR	NA	Dynamic whole-body
Chang and Barrow (1984) **Rat**	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry and colorimetric method	NR	NA	Dynamic head-only

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Fujimaki et al. (2004b) **Mouse** [Exposure parameters in Fujimaki et al. (2004a)]	Paraformaldehyde	NR (Secondary source not found)	Formaldehyde monitor	NR	NA	Dynamic whole-body
Holmstrom et al. (1989a) **Rat**	Paraformaldehyde	Thermal depolymerization	NR	Reported	NA	Dynamic whole-body
Horton et al. (1963) **Mouse**	Paraformaldehyde	Thermal depolymerization	Method of Goldman and Yagoda (reference provided)	NR	NA	Dynamic whole-body
Ito et al. (1996) **Rat**	Formalin w/13% methanol A methanol control group was used	Formalin was placed in 50°C diffusion tubes	4-amino-3-hydrazino-5-mercapto-1,2,4-triazole method; analytical method for methanol NR	Reported NR for methanol	NA	Dynamic (not described)
Kulle and Cooper (1975) **Rat**	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	NR	NA	Dynamic olfactometer
Lang et al. (2008) **Human**	Paraformaldehyde (and ethyl acetate as a masking agent)	Thermal depolymerization	Dinitrophenylhydrazine and HPLC analysis Formaldehyde monitor	NR	NA	"Quasi static conditions"
Meng et al. (2010) **Rat**	Paraformaldehyde	Thermal depolymerization	IR Spectrophotometry	NR	NA	Dynamic (not described)
Moeller et al. (2011) **Monkey**	$[^{13}CD_2]$-formaldehyde	NR	NR	Reported	NA	Dynamic whole-body
Monticello et al. (1989) **Monkey**	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	NR	NA	Dynamic whole-body
Morgan et al. (1984) **Frog**	Paraformaldehyde An ex vivo study of frog palates exposed to formaldehyde gas	Thermal depolymerization	IR spectrophotometry and colorimetric assay	Within 20% of nominal	NA	This is not an inhalation chamber study

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Nielsen et al. (1999) **Mouse**	Paraformaldehyde	Thermal depolymerization	NR	NR	NA	Dynamic whole-body
Morgan et al. (2017) **Mouse**	Paraformaldehyde	Thermal depolymerization	Formaldehyde meter	NR	NA	Dynamic whole-body
Ozen et al. (2003a) **Rat**	Paraformaldehyde	Thermal depolymerization	Formaldehyde monitor	NR	NA	Dynamic whole-body
Ozen et al. (2003b) **Rat**	Paraformaldehyde	Thermal depolymerization	Gas chromatography and formaldehyde monitor	NR	NA	Dynamic whole-body
Ozen et al. (2005) **Rat**	Paraformaldehyde	Thermal depolymerization	Formaldehyde monitor	NR	NA	Dynamic whole-body
Sari et al. (2004) **Mouse**	Paraformaldehyde	NR (Secondary source not found)	"a chemical method" and Formtector XP-308	Reported	NA	Dynamic whole-body
Sari et al. (2005) **Mouse** Cited exposure parameters from Sari et al. (2004)	Paraformaldehyde (Mice were exposed intranasally to 500 ppm toluene/mouse 6 hr/d for 3 da prior to formaldehyde exposure)	NR (Secondary source not found)	"measured chemically" and Formtector XP-308	Reported	NA	Dynamic whole-body
Sari et al. (2005) **Mouse**	Paraformaldehyde	NR (Secondary source not found)	"measured chemically" and Formtector XP-308	Reported	NA	Dynamic whole-body
Sarsilmaz et al. (1999) **Rat**	Paraformaldehyde	Thermal depolymerization (reference provided)	Formaldehyde monitor	NR	NA	Dynamic whole-body
Sarsilmaz et al. (2007) **Rat** [Assumed to be the same cohort as Aslan et al. (2006)]	Paraformaldehyde	Thermal depolymerization (reference provided)	Formaldehyde monitor	NR "Desired concentrations were prepared"	NA	Dynamic "prism-shaped glass covers"
Schachter et al. (1986) **Human**	Paraformaldehyde (apparent co-exposure to 2-propanol)	Thermal depolymerization over boiling 2-propanol	Chromotropic acid	Reported	NA	Dynamic whole-body

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Schachter et al. (1987) **Human**	Paraformaldehyde (apparent co-exposure to 2-propanol)	Thermal depolymerization over boiling 2-propanol	Chromotropic acid	Reported	NA	Dynamic whole-body
Songur et al. (2005) **Rat**	Paraformaldehyde	Thermal depolymerization	Formaldehyde monitor	NR	NA	Dynamic
Sorg et al. (1998) **Rat**	Paraformaldehyde	Thermal depolymerization	HPLC	Reported 44% decline in concentration over the course of the experiment	NA	Dynamic whole-body
Sorg et al. (2001b) **Rat** *Experiment 2 and 3* *(See also Experiment 1-**Inadequate**)*	Paraformaldehyde	Thermal depolymerization	HPLC (Sorg et al., 1998)	NR	NA	Dynamic whole-body
Sorg et al. (2004) **Rat**	Paraformaldehyde with co-exposure to orange oil (a known irritant)	Thermal depolymerization	Photoacoustic multi-gas monitor	Reported	NA	NR
Sorg and Hochstatter (1999) **Rat** *Experiment 2* *(See also Experiment 1-**Inadequate**)*	Paraformaldehyde	Thermal depolymerization	HPLC (Sorg et al., 1998)	NR	NA	Dynamic whole-body
Wilmer et al. (1987) **Rat**	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	NR	NA	Dynamic whole-body
Wilmer et al. (1989) **Rat**	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	NR	NA	Dynamic Whole-body
Witek et al. (1986) **Human**	Paraformaldehyde (apparent co-exposure to 2-propanol)	Thermal depolymerization over boiling 2-propanol (82.5°C)	Chromotropic acid	Reported	NA	Dynamic whole-body
Witek et al. (1987) **Human**	Paraformaldehyde (apparent co-exposure to 2-propanol)	Thermal depolymerization over boiling 2-propanol (82.5°C)	Chromotropic acid	Reported	NA	Dynamic whole-body

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Poor Exposure Characterization: there are serious uncertainties or limitations regarding exposure methodology.						
Al-Saraj (2009) **Rabbit**	10% Formalin No methanol control [Pretreatment with Ivermectin which can cause cleft palate and clubbed forelimbs in rabbits]	Evaporation	Colorimetric method (based on a reference) Methanol not measured	Reported (12 ppm)	NA	Dynamic whole-body
Amdur (1960) **Guinea pig**	Formalin (37%)	Sintered glass bubbler	Colorimetric method and chromotropic acid	Reported	NaCl particles measured	Dynamic whole-body
Arican et al. (2009) **Rat**	Paraformaldehyde	Thermal depolymerization	NR	NR	NA	Dynamic whole-body
Bansal et al. (2011) **Rabbit**	10% Formalin 40% Formalin No methanol control	Evaporation from open containers	NR	NR Target and nominal concentrations also NR	NA	Open containers of formalin were placed below cages
Biagini et al. (1989) **Monkey**	Formalin w/10-15% methanol No methanol control [Anesthesia with ketamine and xylazine, which cause bronchodilation, could affect pulmonary function measurements.]	Injected into a GC injector and heated to 220-230°C	Formaldehyde monitor Methanol not measured	Reported	NA	Dynamic whole-body
Bian et al. (2012) **Rat**	Formalin No methanol control	Evaporation	Formaldehyde meter Methanol not measured	10.0 ± 1.0 mL/m^3	NA	Dynamic whole-body
Bhalla et al. (1991) **Rat**	Paraformaldehyde	Thermal depolymerization	NR	NR	NA	Dynamic nose-only
Bokina et al. (1976) **Rabbit**	NR No methanol control	NR	NR	NR	NA	NR

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Buckley et al. (1984) **Mouse**	Formalin (co-exposure to methanol) No methanol control	NR	IR spectrophotometry Methanol not measured	Reported	NA	Dynamic whole-body
Casset et al. (2006) **Human**	Formalin (35% aqueous medicinal solution of formaldehyde; co-exposure to methanol) No methanol control	Evaporated from a Pyrex boiler at 85°C	HPLC Methanol not measured	<10% of target	NA	Dynamic whole-body with subjects wearing masks
Chonglei et al. (2012) **Mouse**	Mice were simultaneously exposed to formaldehyde, benzene, toluene, and xylene vapors. The test article for formaldehyde was NR	NR	Digital electrochemical analyzer and gas chromatography	NR	NA	Dynamic whole-body (airflow not reported)
Cometto-Muñiz et al. (1989) **Human**	NR No methanol control	NR	Chromotropic acid	Reported	NA	Dynamic olfactometer
Day et al. (1984) **Human**	Solution of formalin in methanol. No methanol control	Atomized and then evaporated on a hot plate.	Chromotropic acid Methanol not measured	Reported	NA	Dynamic whole-body
de Ceaurriz et al. (1981) **Mouse**	NR No methanol control	NR	Colorimetric method Methanol not measured	NR	NA	Dynamic whole-body
Dinsdale et al. (1993) **Rat** *Experiment 1* *(See also Experiment 2 -* ***Robust****)*	Formalin (co-exposure to methanol) No methanol control	Jet atomizer (Exp 1)	IR spectrophotometry Methanol not measured	Reported	NA	Dynamic whole-body
Ezratty et al. (2007) **Human**	Formalin (co-exposure to methanol) No methanol control	Thermal depolymerization	Semiconductor gas sensor Methanol not measured	NR	NA	Dynamic whole-body

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Falk et al. (1994) **Human**	Formalin (co-exposure to methanol) No methanol control.	Evaporation from a heated glass surface	Liquid chromatography	Reported for treated and negative control groups	NA	Dynamic Whole-body
Gieroba et al. (1994) **Rabbit**	***38% Formalin*** No methanol control	Evaporation	None	NR	NA	A tube delivered FA vapor to rabbits' nares
Gofmekler (1968) **Rat**	NR No methanol control	NR	NR Methanol not measured	NR	NA	NR
Gofmekler and Bonashevskaya (1969) **Rat**	NR No methanol control	NR	NR Methanol not measured	***NR***	NA	NR
Golalipour et al. (2007) **Rat**	NR but exposure would have been to formalin with co-exposure to methanol No methanol control	NR, but formaldehyde and methanol would have off-gassed from necropsy tubs of formalin	Formaldehyde Draeger tubes Methanol not measured	Reported	NA	Not a chamber study; rats exposed in dissection room
Guseva (1973b) **Rat**	NR No methanol control	NR Rats were simultaneously exposed by inhalation and drinking water	Fuchsin sulfurous acid method Methanol not measured	NR	NA	Dynamic (not described)
Han et al. (2015) **Rat**	NR No methanol control	NR	NR Methanol not measured	NR	NA	***Static***
Harving et al. (1990) **Human**	Alkaline solution of formalin; co-exposure to methanol No methanol control	Thermal depolymerization	Acetylacetone Methanol not measured	Reported	NA	Dynamic whole-body
Silva Ibrahim et al. (2015) **Rat**	Formalin (purity NR) A vehicle control group was exposed to water No methanol control	Ultrasonic nebulizer	NR	NR	0.5-1 µm MMAD NR	Dynamic whole-body

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Ionescu et al. (1978) **Rabbit**	NR (probably aerosolized formalin) No methanol control	NR	NR Methanol not measured	NR (target and nominal concentrations also NR)	NA	Static
Jaeger and Gearhart (1982) **Mouse and Rat**	Formalin No methanol control	Aerosolization and evaporation	IR spectrophotometry and colorimetric method Methanol not measured	Reported	NA	Dynamic whole-body (Mason jar)
Kamata et al. (1996b) **Rat**	Formalin (with 10% methanol) No methanol control	Sprayed into a bottle heated to 70°C	Acetylacetone Methanol not measured	Reported	NA	Dynamic whole-body
Kamata et al. (1996a) **Rat**	Formalin with 10% methanol No methanol control	Sprayed into a bottle heated to 70°C	Acetylacetone Methanol not measured	Reported	NA	Dynamic nose-only
Kane and Alarie (1977) **Mouse**	Formalin No methanol control	Evaporation	Colorimetric method Methanol not measured	Reported	NA	Dynamic head-only
Katsnelson et al. (2013) **Rat**	NR No methanol control	NR	NR Methanol not measured	Reported	NA	Dynamic whole-body
Kimura et al. (2010) **Rat**	37% Formalin with 15% methanol No methanol control	Dynamic gas generator (evaporation)	4-amino-3-hydrazino-5-mercapto-1,2,4-triazole method Methanol not measured	NR	NA	Dynamic whole-body
Kim et al. (2013b) **Mouse**	NR No methanol control	NR	HPLC	NR	NA	NR
Kitaev et al. (1984) **Rat**	NR No methanol control	NR	Gravimetric (not described) Methanol not measured	NR	NA	Dynamic (not described)
Krakowiak et al. (1998) **Human**	10% Formalin No methanol control	Evaporation	Chromotropic acid Methanol not measured	Reported	NA	Dynamic whole-body
Kum et al. (2007a) **Rat**	Formalin No methanol control	NR	Gas detection pump (reference provided) Methanol not measured	***NR***	NA	Dynamic whole-body

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Lee et al. (1984) **Guinea pig**	4% Formalin w/1% methanol 37% formalin w/10% methanol No methanol control	Aerosol generated by a nebulizer	Formaldehyde: chromotropic acid Methanol: IR spectrophotometry	NR for formaldehyde or methanol	NR	Dynamic whole-body
Liao et al. (2010) **Rat**	Formalin No methanol control	NR	Formaldehyde meter Methanol not measured	NR	NA	Static
Lino dos Santos Franco et al. (2006) **Rat**	Formalin (diluted to 1%; with 0.32% methanol) A methanol control group was used.	Ultrasonic nebulizer	NR for formaldehyde or methanol	NR for formaldehyde or methanol (nominal concentration NR)	NR	Dynamic whole-body
Lino dos Santos Franco et al. (2009) **Rat**	Formalin No methanol control	Ultrasonic nebulizer	NR	NR Methanol not measured	NR	Dynamic (probably whole-body)
Lino-Dos-Santos-Franco et al. (2011b) **Rat**	Formalin (diluted to 1%; with 0.32% methanol) No methanol control	Ultrasonic nebulizer	NR	NR Methanol not measured	NR	NR
Liu et al. (2009a) **Rat**	Formalin (37%) No methanol control	Evaporation from the inner walls of the static chamber	Formaldehyde monitor	Reported	NA	Static
Liu et al. (2010) **Rat**	Formalin (37%) No methanol control	Evaporation from the inner walls of the static chamber	Formaldehyde monitor	Reported	NA	Static
LICM (2006) **Mouse**	Wood baseboard (not described); co-exposure to unidentified chemicals	NR	NR	NR	NA	Dynamic Not described
Maiellaro et al. (2014) **Rat**	Formalin (source and purity NR) The vehicle control was exposed to water	Ultrasonic nebulizer	NR Methanol not measured	NR Note: one exposure level tested	Reported	Dynamic

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Malek et al. (2003c) Malek et al. (2003a) Malek et al. (2003b) **Rat**	Formalin No methanol control	Evaporation from a dish in the chamber	Formaldehyde Draeger tubes Methanol not measured	Reported	NA	Static with holes
Malek et al. (2004) **Mouse**	Formalin No methanol control	Evaporation from a dish in the chamber	Formaldehyde Draeger tubes Methanol not measured	Reported	NA	Static with holes
Maronpot et al. (1986) **Mouse**	Formalin (9.2%w/v) No methanol control	Nebulization and evaporation	Chromotropic acid	Reported	NA	Dynamic whole-body
Matsuoka et al. (2010) **Mouse**	Formalin No methanol control	Evaporation	Cosmos® smell sensor	NR	NA	Dynamic whole-body
Monfared (2012) **Mouse**	NR No methanol control	NR	NR	NR	NA	Dynamic whole-body
Morgan (1983) **Rat**	Paraformaldehyde (reference provided)	Thermal depolymerization	NR	NR	NA	Dynamic whole-body
Nalivaiko et al. (2003) **Rabbit**	Paraformaldehyde	Thermal depolymerization	None	NR	NA	A tube delivered formaldehyde vapor to rabbits' nares
Ohtsuka et al. (1997) **Rat**	NR No methanol control	Aerosol generated by an atomizer	NR Methanol not measured	NR	NR	Dynamic whole-body "test room"
Ohtsuka et al. (2003) **Rat**	1% Formalin No methanol control	Aerosol generated by an atomizer	NR Methanol not measured	NR	NR	Dynamic whole-body "test room"
Pazdrak et al. (1993) **Human**	NR No methanol control	NR	IR spectrophotometry	Reported	NA	Dynamic whole-body
Pitten et al. (2000) **Rat**	Formalin No methanol control	Evaporation from a dish in the chamber	Acetylacetone method and photometric evaluation Methanol not measured	Reported	NA	Static

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Pross et al. (1987) **Human**	Formalin No methanol control	Evaporation of formalin aerosol	Formalin: chromotropic acid Methanol not measured	NR	NA	Dynamic whole-body
Pross et al. (1987) **Human**	Milled UFFI particles (4 µm) contaminated with heavy microbial growth	UFFI aerosol generation not described	UFFI aerosol: gravimetric filters and an aerodynamic particle sizer	NR	NA	Dynamic whole-body
Pross et al. (1987) **Human**	UFFI off-gas products.	UFFI off-gas generated by passing air through beds of fractured UFFI wetted with water	NR	NR	NA	Dynamic whole-body
Pushkina et al. (1968) **Rat**	NR No methanol control	NR	NR Methanol not measured	NR	NA	NR
Sadakane et al. (2002) **Mouse**	Formalin (0.5% solution in saline No methanol control	Aerosol generated by an ultrasonic nebulizer	NR Methanol not measured	NR	NR	NR
Saillenfait et al. (1989) **Rat**	Formalin w/10% methanol No methanol control	Air was bubbled through formalin	IR spectrophotometry Methanol not measured	Reported	NA	Dynamic
Sandikci et al. (2007b) **Rat**	NR No methanol control	NR	NR (reference provided) Methanol not measured	NR	NA	Dynamic whole-body
Sandikci et al. (2009) **Rat**	NR No methanol control	NR	Formaldehyde Draeger tubes	NR	NA	Dynamic whole-body
Sanotskii et al. (1976) **Rat**	NR No methanol control	NR	Colorimetry (not described) Methanol not measured	NR	NA	Dynamic (not described)
Schreiber et al. (1979) **Hamster**	NR No methanol control	NR	NR	NR	NA	NR

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Schuck et al. (1966) **Human**	Formaldehyde and other photooxidation products	Formaldehyde was generated during propylene photooxidation and ethylene photooxidations in a reaction chamber exposed to high intensity UV light (3,000 Å)	Chromotropic acid	Mean concentrations provided in a graph	NA	Reaction chamber with welding masks attached for eye exposure
Senichenkova (1991b) **Rat**	NR No methanol control	NR	Gravimetric (not described) Methanol not measured	NR	NA	Dynamic (not described)
Senichenkova and Chebotar (1996) **Rat**	NR No methanol control	NR	Gravimetric (not described) Methanol not measured	NR	NA	Dynamic (not described)
Sheveleva (1971) **Rat**	NR No methanol control	NR	NR (reference provided); Methanol not measured	Reported	NA	Dynamic whole-body
Sorg et al. (1996) **Rat**	Formalin No methanol control	Air was bubbled through formalin	NR Methanol not measured	Reported	NA	Dynamic whole-body
Sorg et al. (2001b) **Rat** *Experiment 1* *(See also Experiments 2 and 3-**Adequate**)*	Formalin No methanol control	Evaporation of formalin	NR Methanol not measured	NR	NA	Dynamic whole-body
(Sorg et al., 2002) **Rat**	Formalin No methanol control	Evaporation	None	NR	NA	Cotton swabs containing various formalin dilutions were placed in a maze

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Sorg and Hochstatter (1999) **Rat** *Experiment 1* *(See also Experiment 2-* ***Adequate****)*	Formalin No methanol control	Air was bubbled through formalin	NR	NR	NA	Dynamic whole-body
Speit et al. (2011b) **Rat**	Formalin No methanol control	Evaporation	NR Methanol not measured	Reported	NA	Dynamic whole-body
Swenberg et al. (1983b) [book chapter] **Rat and Mouse**	[^{14}C]- formaldehyde	NR	NR	NR	NA	NR
Swenberg et al. (1986) [book chapter] **Rat and Mouse**	NR No methanol control	NR	NR	NR	NA	NR
Tani et al. (1986) **Rabbit**	37% Formalin No methanol control	Evaporation	4-amino-3-hydrazino-5-mercapto-1,2,4-triazole method Methanol not measured	NR	NA	Direct exposure to the upper and lower respiratory tract via two T-tubes
Tepper et al. (1995) **Mouse**	Carpet containing volatile organic compounds, pesticide residues, and microbiological flora	Heating of carpet	Gas chromatography High resolution mass spectrometry	Reported for formaldehyde and 9 other specific organic chemicals	NR	Dynamic head-only
Tarkowski and Gorski (1995) **Mouse**	NR No methanol control	NR	NR Methanol not measured	NR	NA	NR
Wang et al. (2012) **Rat**	NR No methanol control	NR	NR Methanol not measured	NR	NA	Static (not otherwise described)
Weber-Tschopp et al. (1977) **Human**	Formalin (35%) No methanol control	A syringe delivered formalin to a heated (120°C) Pyrex glass tube	Chromotropic acid Methanol not measured	Reported	NA	Dynamic whole-body

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Xing Sy (2007) **Mouse**	NR No methanol control	NR	NR	NR	NA	NR
Yang et al. (2001) **Human**	Plywood (5 layers) which off-gassed formaldehyde and traces of C_6–C_{11} aldehydes.	The plywood was cut into 50- × 10-cm planks and placed in a small chamber to facilitate off-gassing.	Formaldehyde monitor	Reported for formaldehyde, but location of measures NR; concentrations of other gases NR	NA	Eyes were exposed via modified swim goggles
Yorgancilar et al. (2012) **Rat**	NR No methanol control	NR	NR	NR	--	NR
Yu and Blessing (1997) **Rabbit**	38% Formalin No methanol control	Evaporation	None	NR	NA	A tube delivered formaldehyde vapor to rabbits' nares
Yu and Blessing (1999) **Rabbit**	NR No methanol control	NR	None	NR	NA	formaldehyde vapor puffed in front of the rabbits's nares
Zhang et al. (2013) **Mouse**	Formalin (10%) No methanol control	NR	NR	NR	NA	Dynamic nose-only
Zhang et al. (2014b) **Rat**	Formalin No methanol control	Evaporation	NR	Reported but questionable	NA	Static
Zhou et al. (2006) **Rat**	NR No methanol control	NR	Formtector Methanol not measured	NR	NA	NR
Zhou et al. (2011a) **Rat**	NR No methanol control	NR	NR Methanol not measured	NR	NA	Static
Zhou et al. (2011b) **Rat**	NR No methanol control	NR	NR Methanol not measured	NR	NA	Static

HPLC – high performance liquid chromatography; IR – infrared; MMAD (σ_g) – mass median aerodynamic diameter (geometric standard deviation); NA – Not applicable; NR – not reported; PFA – paraformaldehyde.

A.5.2. Sensory Irritation

Literature Search

A systematic evaluation of the literature database on studies examining the potential for sensory irritation in relation to formaldehyde exposure in humans was initially conducted in 2012, with yearly updates to September 2016 (see Section A.5.1). A systematic evidence map identified literature published from 2016 to 2021 (see Appendix F). The search strings used in specific databases are shown in Table A-31. Additional search strategies included:

- A review of reference lists in the the articles identified through the full screening process and
- A review of reference lists in the 2010 draft Toxicological Review for Formaldehyde (U.S. EPA, 2010).

Symptoms of irritation in humans, primarily ocular, nasal, and throat symptoms, were the focus of this review. Inclusion and exclusion criteria used in the screening step are described in Table A-32. The search and screening strategy, including exclusion categories applied and the number of articles excluded within each exclusion category, is summarized in Figure A-22. Based on this process, 58 studies were identified and evaluated for consideration in the Toxicological Review.

Table A-31. Summary of search terms for sensory irritation

Database, search parameters	Terms
PubMed No date restriction	(Formaldehyde[majr] OR paraformaldehyde[majr] OR formalin[majr]) AND (irritation OR irritant OR irritants)
Web of Science No date restriction	TS=(Formaldehyde OR paraformaldehyde OR formalin) AND TS=(irritation OR irritant OR irritants)

Table A-32. Inclusion and exclusion criteria for studies of sensory irritation

	Included	Excluded
Population	• Human	• Animals
Exposure	• Indoor exposure via inhalation to formaldehyde • Measurements of formaldehyde concentration in air	• Not formaldehyde • Dermal • Exposure defined using job title/industry • Outdoor exposure
Comparison	• Evaluated health outcomes and associations with formaldehyde exposure	• Case reports • Surveillance analysis /Illness investigation (no comparison)

	Included	Excluded
Outcome	• Ocular, nasal and throat symptoms	• Exposure studies/no outcome evaluated • Studies evaluating other health outcomes • Properties, uses
Other		• Reviews and reports (not primary research), letters, meeting abstract, no abstract, methodology paper, nonessential article in a foreign language

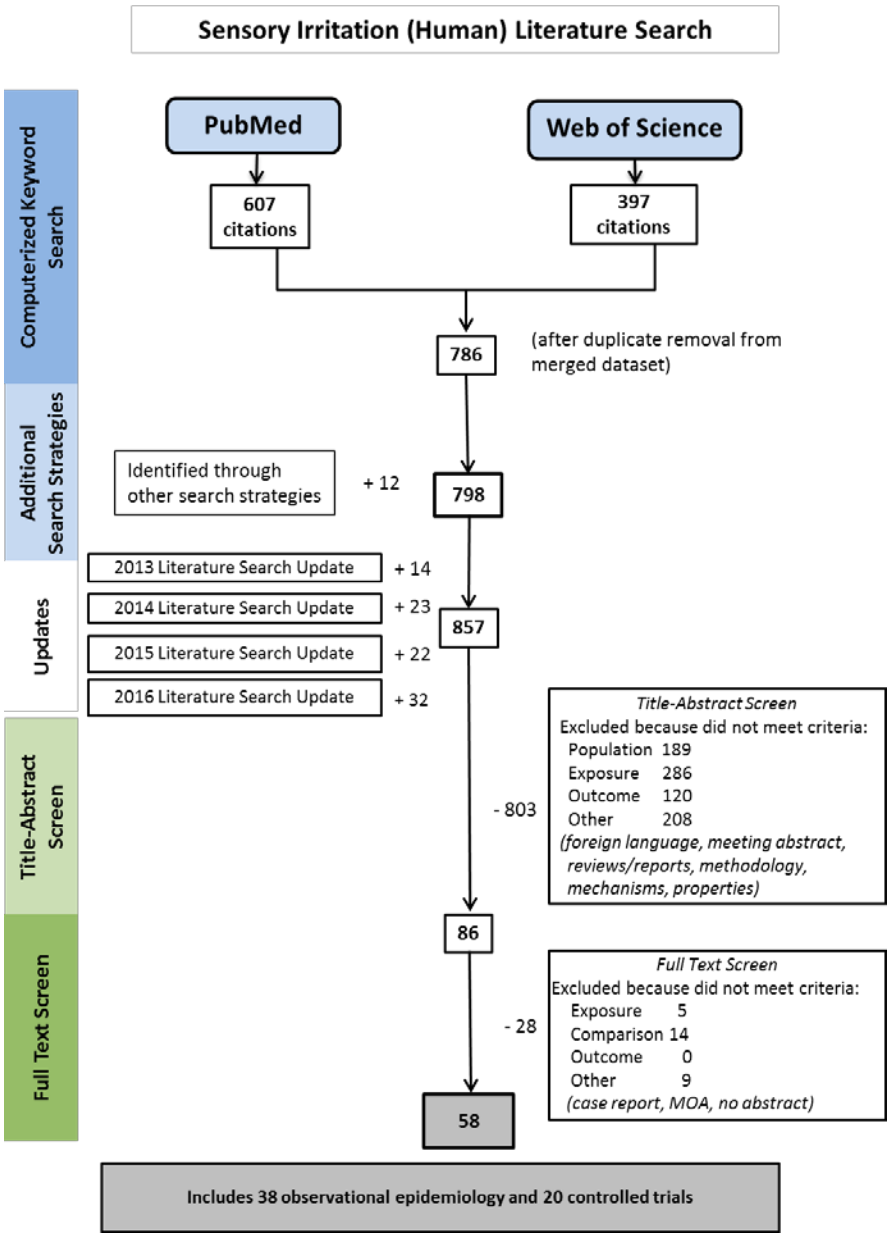


Figure A-22. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and sensory irritation in humans.

Study Evaluations

All articles identified for consideration in the literature search for sensory irritation were evaluated to determine the degree of confidence in the reported results regarding the association of formaldehyde inhalation with sensory irritation in humans. Observational epidemiology and controlled human exposure studies were evaluated. The results of controlled human exposure studies were considered to be relevant to the health assessment because irritation appears to be an

acute phenomenon rather than a time-dependent chronic response. Each study was evaluated for precision and accuracy of exposure assessment, measurement of outcome, participant selection and comparability, possibility of confounding, analysis and completeness of results, and study size. Table A-33 provides criteria used to categorize the epidemiology studies. The accompanying tables in this section document the evaluation. Studies are arranged alphabetically within each table.

Symptoms related to irritation in the eyes, nose, and throat were reported by most studies. Generally, symptoms were ascertained via self-report or through interviews, both using a standardized questionnaire (e.g., American Thoracic Society [ATS]). Generally, self-reported symptoms will be influenced to some degree by recall bias if exposure is known to the responder, although this is of less concern if an appropriate comparison is used. For some studies, there were more serious concerns about selection or information bias related to the participants' knowledge of their exposure or selection into a study based on presence of symptoms and concerns about exposure, which could produce spurious findings (Salonen et al., 2009; Wei et al., 2007; Ritchie and Lehnen, 1987; Bracken et al., 1985; Norsted et al., 1985; Ritchie and Lehnen, 1985; Dally et al., 1981).

The time frame of the exposure assessment relative to the assessment of symptoms was an important aspect of the evaluation of symptom prevalence. Questions about symptom occurrence over an extended time period (weeks and months) that were separated in time from the exposure assessment period were considered to be more limited by recall bias. This limitation was apparent in some of the studies of anatomy students. The occupational studies generally ascertained the prevalence of symptoms while at work via interview using standardized questionnaires.

Treatment of potential confounding by studies also was evaluated. EPA considered age, gender, and smoking to be important confounders to evaluate for effects on sensory irritation. EPA also looked for consideration of confounding by other irritants in the workplace, depending on the occupational setting.

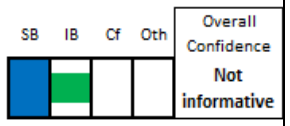
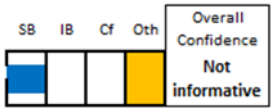
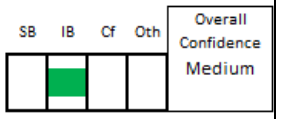
Table A-33. Criteria for categorizing study confidence in epidemiology studies of sensory irritation

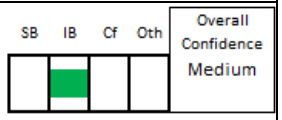
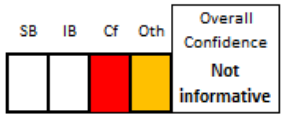
Confidence	Exposure	Study design and analysis
High	**General population:** Exposure measure corresponds to appropriate time window for outcome ascertainment (e.g., measures in more than one season if time window covers 12 months or addressed season in the analysis). Exposure assessment designed to characterize mean individual exposures appropriate to analysis. **Work settings:** Ability to differentiate between exposed and unexposed, or between low and high exposure.	Instrument for data collection (e.g., ATS questionnaire) described or reference provided. Symptoms reported without knowledge of exposure status. Assessment of symptoms timed concurrent with exposure assessment. Analytic approach evaluating dose-response relationship using analytic procedures that are suitable for the type of data, and quantitative results provided. Confounding considered and addressed in design or analysis; large sample size (number of cases).

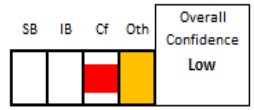
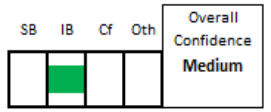
Confidence	Exposure	Study design and analysis
Medium	**General population:** More limited exposure assessment, or uncertainty regarding correspondence between measured levels and levels in the etiologically relevant time window. **Work settings:** Referent group may be exposed to formaldehyde or to other exposures affecting respiratory conditions (potentially leading to attenuated risk estimates)	Instrument for data collection less well described. Symptoms reported without knowledge of exposure status. Assessment of symptoms timed concurrent with exposure assessment. Analytic approach more limited; confounding considered and addressed in design or analysis but some questions regarding degree of correlation between formaldehyde and other exposures may remain. Sample size may be a limitation.
Low	**General population:** Short (<1 d) exposure measurement period without discussion of protocol and quality control assessment.	High likelihood of confounding that prevents differentiation of effect of formaldehyde from effect of other exposure(s), limited data analysis (or analysis that is not appropriate for the data) or small sample size (number of cases).
Not informative	Exposure range does not allow meaningful analysis of risks above 0.010 mg/m^3; no information provided.	Concern regarding selection bias with direction away from null. Description of methods too sparse to allow evaluation.

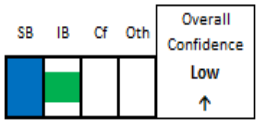
Controlled human exposure studies were evaluated for important attributes of experimental studies, including randomization of exposure assignments, blinding of subjects and investigators, and inclusion of a clean air control exposure and other aspects of the exposure protocol. The evaluation of few individuals ($n \leq 10$) resulted in reduced confidence. Several studies did not describe the measures used to control bias, resulting in a lower level of confidence in study results. However, some of these studies evaluated multiple dose levels, an important strength for the hazard assessment. Therefore, these studies were included with medium confidence when reporting detail was the only identified limitation.

Table A-34. Evaluation of studies examining sensory irritation in humans: residential studies

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Bracken et al. (1985) (Ontario) Residential (prevalence)	Exposed homes randomly selected from a group currently being monitored for formaldehyde and previously at homeowner request. Possible selection bias.	Area samples; average of 3 hr samples; approx. 5 per home. UFFI Mean 0.07, max 0.13 mg/m^3; non-UFFI Mean 0.06, max 0.12 mg/m^3; Lab Mean 0.15, max 7.2 mg/m^3. Limited sampling period, details of sampling protocol not provided. Most samples may have been below LOD (NIOSH, 1977, chromotropic)	Self-report, ATS question-naire. Response was not blinded to presence of UFFI.	Exposed: Homes with UFFI, Referent: non-UFFI homes from university community; age and smoking prevalence similar.	Symptom prevalence estimated from graphs in Figures 1 and 2 in publication. Compared prevalence by exposure group, *t*-test	N = 54 exposed; N = 26 referent	 Selection bias probable; formaldehyde concentration similar in comparison groups
Dally et al. (1981) (Wisconsin) Residential (prevalence)	Survey of homes reported to State Division of Health because of symptoms; potential for selection bias	Area samples; average of 30–60 min samples in multiple locations. LOD 0.12 mg/m^3 Mobile homes, Median 0.58, range <0.12 to 4.53 mg/m^3. Conventional, Median 0.12, range <0.12 to 1.34 mg/m^3. Limited sampling period.	Self-report, questionnaire. Responses blind to formaldehyde measurements.	No comparison group; smoking status was not associated with formaldehyde concentration; no adjusted results provided	Symptom prevalence among exposed	N=256	 No comparison group; potential for selection bias; limited statistical analyses
Hanrahan et al. (1984) (Wisconsin) Residential (prevalence)	Recruited from a randomly selected list of mobile homes in Wisconsin; response rate 31%. Concern is less because formaldehyde concentrations, age,	Area samples; average of 1 hr samples from 2 rooms. Median 0.2 mg/m^3, range <0.12 to 0.98 mg/m^3 Limited sampling period in closed residence with no point formaldehyde emissions; sampling and	Self-report, questionnaire, no description Response blind to formaldehyde	Logistic regression adjusting for age, gender, and smoking status.	Logistic regression, provided graph of predicted mean prevalence normalized to mean age, and upper and lower 95% CI by concentration from regression model	N = 61	 Limited sampling period; Questionnaire not described.

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	and gender were comparable to nonrespondents, and participants blinded to formaldehyde concentration.	analytic protocols referenced; LOD 0.12 mg/m^3	measurements.				
Liu et al. (1991); Sexton et al. (1986) (California) Residential (prevalence)	Recruited from a randomly selected, age-stratified list of mobile homes in California; response rate 44%. However, the proportion of respondents with asthma was not different from U.S. prevalence in the 1980s (4.7% age-adjusted; MMWR Surveillance Summaries; April 24, 1998 / 47(SS-1);1-28), suggesting minimal concern for selection bias.	Area samples using passive monitors; 7-d average in 2 rooms in 2 seasons. Mean summer 0.089 ppm, winter 0.088 ppm; TWA concentration estimated using average concentration multiplied by # hours spent in the home per day during the week of sampling. Validity study (Sexton et al., 1986) reported LOD of 0.01 ± 0.30 ppm; range, LOD - 0.57 mg/m^3	Self-report, mailed questionnaire, no description. Responses blind to formaldehyde measurements. Appropriate time frame relative to exposure measurements.	Logistic regression adjusting for age, gender, smoking status, status of chronic respiratory disease/allergy.	Logistic regression, beta coefficients for change in symptom prevalence per concentration change were not provided. Prevalence estimated from graph of prevalence by category of formaldehyde TWA exposure in publication.	836 homes, 1,096–1,394 individuals	 Questionnaire not described
Lovreglio et al. (2009) (prevalence)	Selection of 59 homes in city not described.	24 hr samples in kitchen in 59 homes; reported mean, median, range.	Self-report, questionnaire (onset of symptoms while in kitchen).	Formaldehyde and acetaldehyde concentrations were correlated (p = 0.001). Formaldehyde concentrations varied by smoking status. Data analyses	No data provided, qualitative results only.	182 subjects living in 59 homes	 Results of data analysis were not provided; confounding by smoking or co-exposure was not addressed

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	consideration of likely confounding	Analysis and completeness of results	Size	Confidence
				not described, no adjustment or stratification.			
Main and Hogan (1983) (prevalence)	Recruitment and selection were not described.	Three 1-hr area samples using impingers taken on 4 occasions (August, September, December, April) always on a Monday. At least 1 sample was taken from each office in both trailers. Limited sampling period in closed residence with no point formaldehyde emissions; sampling and analytic protocols referenced; referent group assumed to have no exposure. 0.15–1.97 mg/m^3	Self-report, ATS question-naire, symptom history at work	Potential dissimilarity of administrative employees and police officers (healthier); direction of bias possibly away from null; more exposure to ETS among referent; possible direction toward null	Symptom prevalence at work compared between exposed and referent, chi-square; small sample size	Exposed 21, Referent 18	 Potential dissimilarity between comparison groups; more exposure to ETS among referent; small sample size
Norsted et al. (1985) (Texas) Residential (prevalence)	Homes selected on request of residents; Possible selection bias.	Sampling protocols not described	Self-report; symptom reports not blind to exposure status	No comparison group; no adjusted results provided	Total # participants in homes unknown.	443 mobile homes	 potential for selection bias; Reporting deficiencies, no comparisons
Olsen and Dossing (1982) (Denmark) Day care center workers in	Recruited from all newly built mobile day care centers in 2 boroughs (n = 7) and 3 referent centers selected at random; response rates 94% exposed,	Area samples; average of 2-hr samples in 2–4 locations, on 1 occasion. Exposed mean 0.43, range 0.24 to 0.55 mg/m^3; referent mean 0.08, range 0.05 to 0.11 mg/m^3; limited sampling	Self-report, questionnai re; linear analogue scale for severity, experience within one	Referent selected from stationary child-care facilities in same residential area. Age and smoking prevalence	Prevalence and severity presented in graphs; comparisons between exposed and referent groups	Exposed = 66; Referent = 26	 Some uncertainties regarding temporal

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	consideration of likely confounding	Analysis and completeness of results	Size	Confidence
mobile homes (prevalence)	76% referent. Responses similar in exposed and referent to 3 questions not expected to be related to formaldehyde.	period in closed residence with no point formaldehyde emissions; sampling and analytic protocols referenced	month; questionnaire described and citation provided	similar in exposed and referent.			concordance of exposure and symptom assessments
Ritchie and Lehnen (1987, 1985) (Minnesota) Residential (prevalence)	Selection into survey at request of family physician; potential for selection bias; however, health responses were blind to sampling results	Area samples; average of 30-min samples in 2 rooms. Bedroom mean: Mobile homes 0.43 mg/m^3, Conventional 0.15 mg/m^3, range 0.012 (LOD) to 6.79 mg/m^3. Limited sampling period in closed residence with no point formaldehyde emissions; sampling & analytic protocols referenced;	Self-report, interview; symptoms same day as exposure measurements, respondents did not know the formaldehyde measurement for their homes	Prevalence stratified by age, gender, and smoking status.	Presented graphs of prevalence by exposure (3 categories); tables of prevalence (SE) by type of home, exposure category, and smoking status	N = 2,000 residents; 891 homes	 Potential for selection bias
Salonen et al. (2009) (Finland) (prevalence)	Building selected because of complaints and symptom reports of occupants; possible selection bias	Area sampling in 20 of 176 buildings selected from database of Finnish Institute of Occupational Health, 2001–2006, N = 1–12 per building; during work hours 9–4 pm for 1–2 hrs. LOD 0.5 ppb Mean 0.011 mg/m^3; Max 0.044 mg/m^3. Limited sampling period.	Self-report, standardized questionnaire	No comparison buildings evaluated. Compared concentrations to recommended indoor limit (RIL)	Presented ratio of average concentration divided by recommended indoor limit (based on RD50 for respiration rate in mouse bioassay and adjustment to 24 hrs based on Haber's Law.	20 buildings	 Possible selection bias; no comparison group

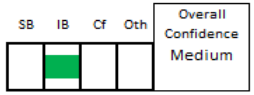
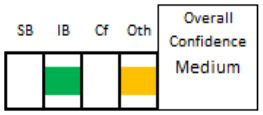
Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Thun et al. (1982) (prevalence)	No information to evaluate	No formaldehyde measurements	Self-report, questionnaire; new symptoms over a 1 yr period.	Exposed: Homes with UFFI, Referent: homes without UFFI. No information to compare exposed and referent	Data were not provided, qualitative results with *p*-values	1,396 exposed, 1,395 referent	 Inadequate reporting detail; no formaldehyde measurements
Zhai et al. (2013) Jan 2008–Dec 2009 (China) (prevalence)	Provided criteria for selection of homes in defined area; evaluated 186 homes in Shenyang, China; homes were decorated in last 4 years and occupied within the last 3 yrs.	Cited Code for indoor environmental pollution control of civil building engineering (GB50325-2001); sampling period not reported. Samplers in breathing zone in bedroom, living room and kitchen; *N* = 558 in 186 homes; exposure groups polluted homes: > 0.08 mg/m^3, mean 0.09–0.13 mg/m^3 in 3 rooms; nonpolluted ≤0.08 mg/m^3, mean 0.04–0.047 mg/m^3.	Respiratory symptoms via questionnaire (ATS, 1978); randomly selected one adult from each house, plus 82 children (assisted by parents)	Prevalence ratios for specific symptoms/ disorders unadjusted for other variables, characteristics in two groups not described; regression analyses of combined respiratory symptoms were adjusted	Compared symptom prevalence for children and adults by exposure category (reported *p*-values); multivariate logistic regression of respiratory system symptoms (all) in children and adults, adjusting for age, gender, smoking in family, occupation, education, ventilation frequency, domestic pets, house facing, family history of allergy, height, weight.	Polluted homes *N* = 119; Nonpolluted homes N = 67	Symptom prevalence ratios  Sampling period not reported Analysis of combined respiratory symptoms 

Table A-35. Evaluations of studies examining sensory irritation in humans: school-based studies

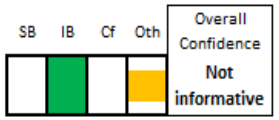
Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Wantke et al. (1996b) (Austria) Schools (panel, intervention)	Children at school where symptoms were reported; evaluated all children attending 3 forms; low concern for selection	Area samples; Sample number and duration not described; s.d. not reported. Concentration in 3 grades: Before move: 0.053, 0.085, 0.092 mg/m^3; After move: 0.036, 0.028, 0.032 mg/m^3	Symptoms assessed before and 3 mos after a move to a different school building. Symptoms reported by parents in a standardized questionnaire. Participants and investigators not blinded.	Comparison to self before and after removal from exposure	Symptom prevalence before and after move; McNemar test of difference	*N* = 62	 Participants and investigators not blinded; Reporting deficiencies

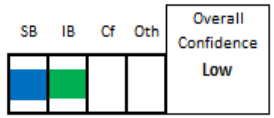
Table A-36. Evaluations of studies examining sensory irritation in humans: controlled human exposure studies

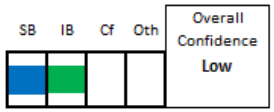
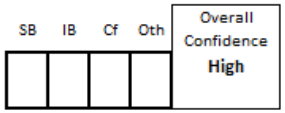
Reference	Exposure assessment (quality descriptor and exposures)	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure)	Consideration of likely confounding	Results presentation	Size
Andersen and Molhave (1983); Andersen (1979) Confidence: Medium	Paraformaldehyde, dynamic chamber, analytical concentrations reported; 0.24, 0.4, 0.81, 1.61 mg/m^3	Self-report, questionnaire; symptom scores	Random assignment to order of exposure, blinding not described. 31.2% smokers.	Within person comparison	Provided prevalence	*N* = 16
Bender et al. (1983) Confidence: Low	Paraformaldehyde, dynamic chamber, analytical concentrations not reported; 0, 0.43, 0.69, 0.86, 1.11, 1.23 mg/m^3	Self-report response (eye only), time to 1st response	Order of exposure assignment not described, blinding not described	Within person comparison	Provided prevalence	*N* = 7

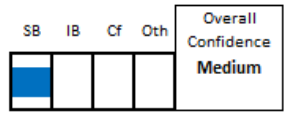
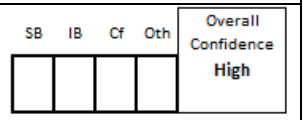
Reference	Exposure assessment (quality descriptor and exposures)	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure)	Consideration of likely confounding	Results presentation	Size
Berglund et al. (2012) Confidence: High	Paraformaldehyde, analytical concentrations reported; series of 18, 0.0078–1.23 mg/m^3;	Nasal irritation (< 3 sec sniffs); Self-report, forced choice response	Exposure concentrations randomly presented; blinding not described.	Within person comparison	Graph of detection prevalence by ln concentration	*N* = 31
Day et al. (1984) Not informative	Marginal; no clean air exposure, 1.23 mg/m^3	Self-report, questionnaire	Nonrandom exposure assignment, blinding not described	No comparisons	Provided prevalence	*N* = 18
Green et al. (1987) Confidence: HIgh	Paraformaldehyde, dynamic chamber, analytical concentrations reported; 0, 3.69 mg/m^3	Self-report, questionnaire; symptom scores	Random assignment to order of exposure, single blinded.	Within person comparison	Provided prevalence & statistical analyses	*N* = 22
Green et al. (1989) Confidence: High	Paraformaldehyde, dynamic chamber, analytical concentrations reported; 0, 3.69 mg/m^3	Self-report, questionnaire; symptom scores	Random assignment to order of exposure, double blinded.	Within person comparison	Provided score data and statistical analyses graphically	*N* = 24
Krakowiak et al. (1998) Not informative	Formalin, no methanol control; analytic concentrations reported; 0.5 mg/m^3	Self-report, diary; symptom scores	Nonrandom exposure assignment, single blinded.	Within person comparison	Provided average symptom scores	2 groups. *N* = 10 in each
Kulle (1993); Kulle et al. (1987) Confidence: Medium	Paraformaldehyde, dynamic chamber, analytical concentrations reported; I: 0, 0.62, 1.23, 2.46, II: 0, 1.23, 3.69 mg/m^3	Self-report, questionnaire; symptom scores	Random assignment to order of exposure, blinding not described.	Within person comparison	Regression coefficients not provided, only *p*-values	I: *N* =10; II: *N* =9
Lang et al. (2008) Confidence: High	Paraformaldehyde, "quasi-static" chamber conditions, analytical concentrations reported; 0, 0.19, 0.37, 0.62, peaks to 1.23 mg/m^3	Self-report, questionnaire; objective measures	Random assignment to order of exposure, double blinded.	Within person comparison	Graphs/tables and statistical analyses	*N* = 21
Mueller et al. (2012) Confidence: High	Paraformaldehyde, dynamic chamber, analytical concentrations reported; clean air, 0.37 + 4 peaks of 0.74 mg/m^3, 0.49 + 4 peaks of 0.98 mg/m^3, 0.62 mg/m^3 and 0.86 mg/m^3	Self-report, questionnaire; objective measures	Exposure concentrations randomly presented; blinding not described.	Within person comparison	Graphs of difference between pre- and end of test values	*N* = 41

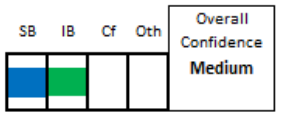
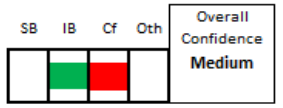
Reference	Exposure assessment (quality descriptor and exposures)	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure)	Consideration of likely confounding	Results presentation	Size
Sauder et al. (1986) Not informative	Paraformaldehyde, dynamic chamber, analytical concentrations reported; 0, 3.69 mg/m^3	Self-report, questionnaire; symptom scores	Nonrandom exposure assignment, blinding not described.	Within person comparison	Provided average symptom scores & statistical analyses	*N* = 9
Schachter et al. (1986); Witek et al. (1986) Confidence: Medium	Paraformaldehyde over boiling 2-propanol, dynamic chamber, analytical concentrations reported	Self-report, questionnaire; symptom scores	Random assignment to order of exposure, double blinded.	Within person comparison	Provided prevalence and score	*N* = 15
Schachter et al. (1987) Confidence: Medium	Paraformaldehyde over boiling 2-propanol, dynamic chamber, analytical concentrations reported.; 0, 2.46 mg/m^3	Self-report, questionnaire; symptom scores	Random assignment to order of exposure, double blinded. Participants had routine occupational formaldehyde exposure, *N* = 2 smokers.	Within person comparison	Provided prevalence and scores	*N* = 15
Schuck et al. (1966) Not informative	Propylene and ethylene photooxidation with UV light; eye exposure only; analytic concentration reported graphically; 0.12−1.23 mg/m^3	Self-report, questionnaire; objective measures	Nonrandom exposure assignment, blinding not described	Within person comparison	Graphs	*N* = 12
Witek et al. (1987); Witek et al. (1986) Confidence: Medium	Paraformaldehyde over boiling 2-propanol, dynamic chamber, analytical concentrations reported; 0, 2.46 mg/m^3	Self-report, questionnaire; symptom scores	Random assignment to order of exposure, double blinded.	Within person comparison	Provided prevalence and score	*N* = 15
Yang et al. (2001) Not informative	Plywood exposure; 2.03, 3.68, 5.3 mg/m^3; eye exposure only; Analytical concentrations reported for formaldehyde but not for other off gassed compounds	Objective measure	Random assignment to order of exposure, double blinded. 25% smokers.	Within person comparison	Graph of eye blink frequency and table of *p*-values	*N* = 8

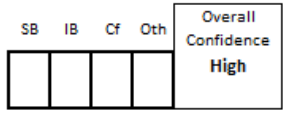
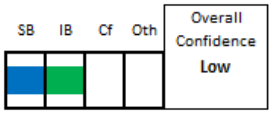
Table A-37. Evaluation of studies examining sensory irritation in humans: anatomy courses

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Akbar-Khanzadeh et al. (1994) (Ohio) Anatomy students (cross-sectional)	Participation not reported.	TWA personal breathing zone samples obtained on all exposed subjects (9 days), and 1 unexposed (6 days). Exposed mean 1.53, range 0.086 to 3.62 mg/m^3. Referent mean 0.12, range 0.09 to 0.17 mg/m^3.	Self-report, Medical Research Council standardized questionnaire	No comparisons reported.	Provided symptom prevalence during exposure, no comparison to baseline or to unexposed; no statistical data analysis	34 exposed; 12 referent	 No within person comparison to baseline or the referent; Reporting deficiencies
Chia et al. (1992) (Singapore) Anatomy students (cross-sectional)	Medical students in 1st year lab course (92% participation); referent group = 3rd or 4th year medical students (participation rate not reported)	Area samples at dissecting tables, n=6, collected on two occasions. Personal samples, n=14 students, duration 2.5 hours; mean 0.91, SD = 0.22 mg/m^3, range 0.50 to 1.48 mg/m^3, LOD = 0.062 mg/m^3. Assumed no formaldehyde exposure in referent based on activities (ward rounds and classroom).	Self-report, modified MRC standardized questionnaire; symptoms during previous 4 wks of course (recall accuracy reduced?)	Comparison to referent matched on age, sex and ethnicity	Symptom prevalence in exposed compared to referent; Referent activities very different	Exposed N = 150; referent N = 189	 Questions about dissimilarity of 1st and 4th year students and potential for recall bias during previous 4 weeks of course

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Fleisher (1987) Anatomy students (cross-sectional)	44% of 204 surveyed in gross anatomy course; of those less than 50% responded to both questionnaires. Greater motivation to participate among those with symptoms?	Area samples in 6 labs, 1 day during semester (approximately 3 hours); Drager tubes, 3 labs, LOD 1.23 mg/m^3, NIOSH method, 3 labs, LOD 0.02 mg/m^3. Personal breathing zone for 2 instructors. 0.64, 0.18 mg/m^3; probable nondifferential misclassification due to sampling method with low sensitivity (3 labs) and low frequency of sampling. Adequate differentiation between exposure groups	Self-report, questionnaire; data collection 1 month after end of course; symptoms all or some of the time, rarely or never. (temporal gap reduced recall accuracy?)	Within person comparison: symptoms during lab with exposure compared to lab with no exposure to formaldehyde.	Compared mean symptom scores, paired *t*-test	N = 38	 Low response to both questionnaires and selection potential; temporal gap in symptom response reduced recall accuracy potential
Kriebel et al. (1993) (Massachusetts) Anatomy students (panel)	96% participation	Personal samples in the breathing zone, 1–1.5 hours; multiple days. Range 0.60–1.14 mg/m^3, geometric mean = 0.9, SD 1.5 mg/m^3	Self-report; questionnaire before, during and immediately after lab each day	Within person comparison: symptoms during and after lab compared to prelab symptoms.	Symptom prevalence before, during and after lab. Mean prelab and cross-lab change over 10 weeks evaluated using multivariate linear regression	N=24	

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Kriebel et al. (2001) (Massachusetts) Anatomy students (panel)	94.4% participation; attendance declined from n=37 to n=10 over 13 wks (better attendance by healthy individuals?)	Individual TWA using zone-exposure matrix based on continuous monitoring in 6 homogenous sampling zones (LOD = 0.06 mg/m^3). 12 min work-zone concentrations calculated using sampling data and recorded work; locations. Mean 1.35, SD 0.69 mg/m^3; 12 min peak 13.42 mg/m^3	Self-report, questionnaire; symptom intensity 10-point scale	Within person comparison: symptoms before and after lab	Generalized estimating equation regression accounting for lack of independence of repeated measures in individuals; symptom intensity, % change per ppm or ppm-weeks	N=38	
Mori et al. (2016) (Japan) Medical students, 1st and 2nd year	Students (2nd year) enrolled in afternoon gross anatomy classes, April–July 2013, mean age 22.9 yrs; compared to nonexposed 1st year students, mean age 21.2 yrs. 75% males	Area sample, 5 locations during class on same day questionnaires were completed. Mean (SD) 0.1 (0.02) ppm	Questionnaire, 16 subjective symptoms, frequency never, sometimes, or often; administered April 2013 before, May 2013 during, and January 2014 6 mos after completion of course.	Presented characteristics by exposure group; adjusted for age, sex and allergy status in regression models.	Prevalence of symptoms compared, Cochran's Q test and McNemar's test; Regression of presence or absense of symptoms in relation to exposure group on day of survey, controlling for doctor-diagnosed allergies, sex and age	123 exposed (98.4%); 114 unexposed (91.9%)	

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Saowakon et al. (2015) (Tailand) Medical students and academic staff	Students and faculty in gross anatomy dissection labs; Selection, recruitment and participation was not reported. Ages 19–21 yrs, nonsmokers with no history of chronic respiratory disease or symptomatic illness	Personal samplers (n=36 students, 4 instructors); area samples, all NIOSH-2016 method; 3-hr samples over duration of class, 3 classes, January, August, and October Students: Mean (SD) ppm Class 1: 0.193 (0.120) Class 2: 0.271 (0.159) Class 3: 0.828 (0.182)	Questionnaire, 20 symptoms, completed before start of dissection and after chest and abdominal opening (classes 2 & 3); Severity scale, 0–4.		Reported each symptom as percentage of score for all symptoms averaged over all classes; no comparisons	N=36 students; n=4 instruc-tors	 No within person comparison to baseline or the referent; reporting deficiencies
Takahashi et al. (2007) (Japan) Medical students (panel)	Did not report # recruited versus # that agreed to complete questionnaire. Not clear if there were refusals.	Area samples in 8 locations in lab, > 10 min; Personal samples (breathing zone) on 18/143 students. Mean 3.0, SD = 0.60 mg/m^3, range 2.2 to 4.6 mg/m^3.	Self-report, questionnaire after 1st day and at end of 2-mo course.	Within person comparison: symptoms after 1st day and at end of course	Symptom prevalence after first day and after lab at end of course; McNemar exact test (estimated from Figure 1 in publication).	N=143	 Large gap between symptom ascertainment and exposure measurements
Takigawa et al. (2005) (Japan) Anatomy students (intervention)	Volunteers; 76% completed questionnaires both before and during lab	Area samples in 9 locations in lab, > 10 minutes. Personal samples on 24 of 78 in phase I (2001) (duration 42–962	Self-report, questionnaire before and during each course; frequency (4-point scale); score	Groups similar in age and % male/female; prevalence of smoking not reported.	Symptom change index, 25 symptoms, by phase of intervention; Mann-Whitney test.	N = 78	

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		minutes); median 3.3 mg/m^3, range 2.2 to 8.9 mg/m^3, and on 46 of 79 in phase II (2004) (duration 100–540 minutes); median 0.88 mg/m^3, range 0.40 to 3.4 mg/m^3.	change during session				
Uba et al. (1989) (California) Anatomy students (panel)	78.6% completed both questionnaires	Personal sampling (impingers) in the breathing zone over 7 mos; multiple days; TWA concentration; range 0.06 to 1.14 mg/m^3	Self-report; American Thoracic Society questionnaire; symptoms after lab on one day in November (at approx. 8–10 wks); symptoms before 1st day and after last day (Sept 1984–Apr 1985)	Within person comparison: persistent symptoms beginning and end of course (7 months); also symptoms during lab session compared to lab with no exposure to formaldehyde.	Numbers with symptoms in exposed and unexposed labs; McNemar's test paired samples, OR, *p*-value.	N=81	
Wantke et al. (1996b) (Austria) Anatomy students (panel)	Volunteers; participation 37.5% (45 of 120 students); possibility of selection bias away from null	Area samples; Continuous daily measurements for formaldehyde at 2 locations during 3-hr lab, 5 d/wk for 4 wks. Mean 0.15, range 0.07 to 0.27 mg/m^3	Self-report, standardized questionnaire at beginning (symptoms during 3 mos before lab) and at end of course (symptoms over last 4 weeks). (recall?)	Within person comparison	Symptom prevalence before and during lab; McNemar exact test; multiple measurements during course would be ideal	N = 45	 Low participation, possibility of selection bias away from null; Potential recall issues – symptoms for previous weeks

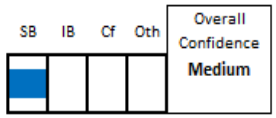
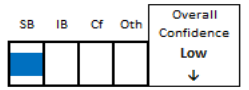
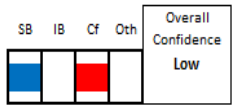
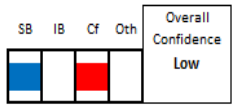
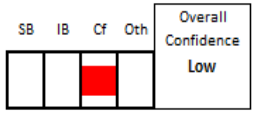
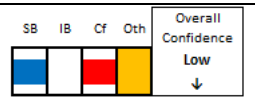
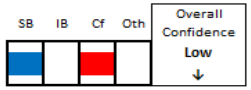
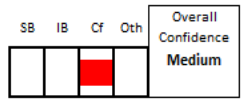
Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Wantke et al. (2000) Austria Anatomy students (panel)	Selection was not described; 27 of the 45 students in Wantke et al. (1996b)	Area samples; Continuous daily measurements for formaldehyde and phenol at 2 locations during lab, exposures for 43 d. Mean 0.27, range 0.13 to 0.41 mg/m^3	Self-report, questionnaire at beginning, 5 wks and 10 wks, Daily symptom cards during class.	Within person comparison; symptoms at beginning and during lab at middle and end of 10-wk course	Symptom prevalence before, middle and at end of 10 wk course; McNemar exact test	N = 27	
Wei et al. (2007) Anatomy students (cross-sectional)	Volunteer, all students present on the day that sampling was conducted; symptom questionnaire was not completed outside of class so difference may have been influenced by perception relative to symptoms in class (possibly resulting in overestimation of risk)	Area samples near dissection tables, 30 min samples, N = 12. Measurements before, beginning, middle and completion of 3-mo gross anatomy class. Geometric mean: before 0.03, beginning 0.89, middle 0.76, end 0.24 mg/m^3 (medium)	Self-report, questionnaire on sampling days after 2 hrs of lab (medium)	Within person comparison (high)	Frequency of symptoms during class; prevalence and severity scores during class compared to "usual life situation"; Walsh test (inadequate comparison)	N = 79–94	

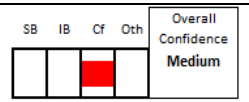
Table A-38. Evaluations of studies examining sensory irritation in humans: occupational studies

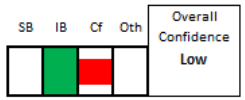
Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Alexandersson et al. (1982) (prevalence)	All exposed workers employed >1 yr; evaluated employees present at work on study day (both exposed and referent); Selection for healthy survivors	TWA personal sampling for formaldehyde, terpenes & dust, N=31; 1 working d, 6–7 hrs 0.05–1.62 mg/m^3; no measurements for referent group; Although no measurements in referent, high concentration in exposed allows assumption of an adequate exposure contrast for comparison of exposed and referent	Self-report, British Medical Research Council questionnaire; symptoms at work, same day as exposure assessment	Symptom prevalence in exposed compared to referent. Exposed: employees of carpentry works; referents were not exposed to formaldehyde or other irritants in same factory; Similar % age, height, sex, & weight. Prevalence smoking 48% in exposed, 40% in referent.	Symptom prevalence at work compared between exposed and referent, chi-square	N=47 exposed; N=20 referent	 Healthy survivor bias
Alexandersson and Hedenstierna (1989) (prevalence, follow-up of Alexandersson et al. (1982)	Evaluated employees who participated in previous study, 4 yr follow-up (Alexandersson et al., 1982); 13 exposed and 2 referents lost-to-follow-up; 13 exposed transferred to unexposed jobs	TWA using personal sampling, 3–4 15 min samples/person; 2 working d; Mean 0.5 mg/m^3; Mean peak 0.69 mg/m^3 limited sampling period; although no measurements	Self-report, British Medical Research Council questionnaire	Symptom prevalence in exposed compared to referent. Exposed: employees of carpentry works; referents were not exposed to formaldehyde or other irritants in same factory;	Change in symptom prevalence at work 1980–1984, chi-square	N=21 exposed; N=18 referent	 Healthy survivor bias; confounding by smoking

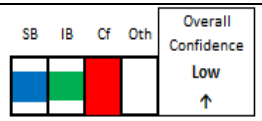
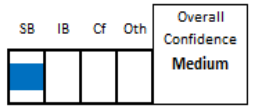
Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	possible survivor bias	in referent, high concentration in exposed allows assumption of an adequate exposure contrast for comparison of exposed and referent		Similar % age, height, sex, & weight. Prevalence smoking 50% in exposed, 33% in referent. Moderate concern for confounding by smoking (direction of bias unclear).			
Alexandersson and Hedenstierna (1988) (prevalence)	Selection for healthy; evaluated employees present at work on study day (both exposed and referent)	TWA using personal sampling, 3–4 15 min samples/person; 1 working d, no concentration reported for referent 0.12–1.32 mg/m^3 Although no measurements in referent, high concentration in exposed allows assumption of an adequate exposure contrast for comparison of exposed and referent	Self-report, standardized questionnaire; outcome assessed same day as exposure	Symptom prevalence among workers exposed to acid-hardening lacquers; referents were "nonexposed" employees at same factory. All male, exposed slightly younger, 50% smokers; referent: 33% smokers. Sampled for dust and solvents: authors considered all exposures to be very low and not confounders. Moderate concern for confounding by	Symptom prevalence at work compared between exposed and referent, chi-square; no adjustment	N=38 exposed; N=18 referent	 Confounding and no adjustment in analyses

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
				smoking (direction of bias unclear).			
Herbert et al. (1994) (prevalence)	Participation >90% in exposed, >80% in referent; Healthy survivor effect likely similar among exposed and referent groups	TWA continuous sample in breathing zone; 5 sites, 2 d 0.09–0.33 mg/m^3 **referent not reported**; sampled for dust. Although no measurements in referent, formaldehyde exposure not expected for oil/ gas field workers, adequate exposure contrast likely for comparison of exposed and referent.	Self-report, Respiratory symptoms ascertained via interview using standardized questionnaire	Possible respiratory irritants in comparison group (oil sands workers); higher prevalence of smokers (52% vs 28%) and shorter duration of employment among exposed, (5 versus 10 yrs)	Symptom prevalence compared by exposure group, chi-square; unadjusted analyses	N=99 exposed; N=165 referent	 Different prevalence smoking and duration of employment between exposed and referent; no adjustment in analyses
Holmström and Wilhelmsson (1988); Wilhelmsson and Holmstrom	100% participation; healthy survivor bias probable; source populations for exposed and referent (government clerks) were different, raising possible unmeasured confounding	Area samples in one group, 1979–1984, personal samples (1–2 hrs) in 1985 in all groups. Sampling data in referent. 0.05–0.5 mg/m^3	Self-report, questionnaire	Groups similar for age and smoking, 87% and 93% male in exposed, 56% male in referent (gender related differences in perception of irritation?) No exposure to	Compared symptoms prevalence across exposure groups, chi-square; unadjusted analyses	N=70 Group 1, N=100 Group 2; N=36 referent	 Healthy survivor bias; groups selected from different source populations; Potential confounding and no adjustment in analyses

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
(1992) (prevalence)		Adequate exposure contrast for comparison of exposed and referent		solvents, concentrations for other chemicals all <1% of OEL (phenol, ammonia, epichlorhydrin, methanol and ethanol).			
Holmström et al. (1991) (prevalence)	Details of recruitment and participation not described. Healthy survivor bias probable; source populations for exposed and referent were different, raising possible unmeasured confounding	Personal exposure measurements stable through year, average 0.2–0.3 mg/m^3, peaks seldom > 0.5 mg/m^3 Formaldehyde Concentration, mean MDF 0.26 mg/m^3, wood dust 0.25 mg/m^3, referent 0.09 mg/m^3; adequate exposure contrast for comparison of exposed and referent	Self-report, questionnaire	MDF group slightly older (44.1 yr) compared to wood (39.3 yr) and referent (39.9 yr); % male varied, smoking less prevalent in referent	Exposed groups each compared to referent; prevalence rate difference, 95% confidence intervals; no adjustment	MDF: N=16 Wood: N=29 Referent: N=36	 Healthy survivor bias; groups selected from different source populations; Potential confounding and no adjustment in analyses
Holness and Nethercott (1989) (prevalence)	Minimal concern for selection bias. Recruitment source was list provided by funeral home	2 area samples (impingers), during embalming, 30 to 180 min.	Self-report, American Thoracic Society questionnaire;	Symptom prevalence compared between exposed (apprentice	Comparisons between exposed and referent, logistic regression adjusted for # pack-	N=84 exposed; N=38 referent	

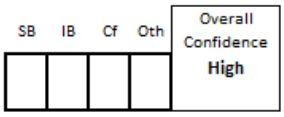
Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	association, 86.6% of eligible participated. Participation rate among referents was not given.	Gave concentration for referent. 0.1–1.0 mg/m^3 Adequate exposure contrast for comparison of exposed and referent	before and after embalming	funeral service workers) and unexposed (service volunteers and paid students), probable unmeasured confounders. Groups similar for age, height, and smoking status. Source of formaldehyde exposure was formalin (also contained methanol)	years smoked. Provided data and results of statistical analyses		Groups selected from different source populations
Horvath et al. (1988) (Wisconsin) Occupational (prevalence)	71% participation in exposed; 88% participation in referent. Age and sex distribution in participants similar to entire workforce in their respective companies. Evaluated and ruled out survivor bias using reasons for leaving employment among 54 former employees; evaluated characteristics of 30/45 nonparticipants	8-hr TWA using Personal and area sampling on day of exam. Exposed mean 1.04, range 0.32 to 4.48 mg/m^3. Referent mean 0.06, range 0.04–0.15 mg/m^3; adequate exposure contrast for comparison of exposed and referent	Self-report, American Thoracic Society questionnaire; assessed same day as exposure assessment; before and after shift	Symptom prevalence in exposed workers at a particleboard manufacturing plant compared to referent workers at 2 food production plants. Higher proportion male in exposed and slightly older average age (expect bias toward null for symptoms). Smoking and mobile home	Symptom prevalence during work in exposed and referent compared; prevalence at end of shift using multiple regression with adjustment	N=109 exposed; N=254 referent	

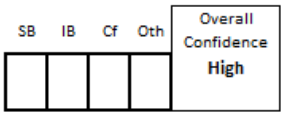
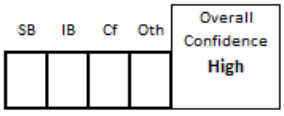
Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	who were younger and higher % male, with similar % smokers and mobile home residency.			residency similar. Particulate exposure in exposed and referent (different sources), other chemical exposures were not detectable or below PEL.			
Kilburn et al. (1985a) (prevalence)	97% participation among exposed.	Environmental samples for formaldehyde, xylene, toluene, and chloroform by regional NIOSH laboratory in 10 of 25 labs; 1–4 hours sampling time; self-report of duration of exposure (hrs/d) 0.25–2.34 mg/m^3; adequate exposure contrast for comparison of exposed and referent	Self-report, questionnaire, composite experience for previous months or years (reduced accuracy of recall, possible recall bias)	Incomplete matching: Among 76 exposed, group of 40 matched to referent on age, cigarette smoking, and ethnicity; multiple chemical exposures; evaluated effects among participants with >4 hrs formaldehyde exposure/d stratified by 2 levels for xylene.	Prevalence by hours formaldehyde exposure and xylene exposure; results of statistical analyses not shown	N=76 exposed; N=56 referent	 Reduced accuracy of recall; incomplete matching
Löfstedt et al. (2011) (prevalence)	>90 % participation in exposed and referent; healthy worker survival? Higher proportion	Individual samples over a single 8-hr shift 0.013–0.19 mg/m^3,	Self-report, questionnaire; existence of symptoms during prior week	Referent from the same industry (not workers in core production or die	Logistic regression models, symptoms by referent, low and high formaldehyde groups; no	N=43 of 48 exposed;	

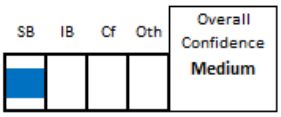
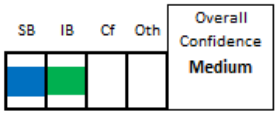
Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	of referents had ever had asthma or allergic symptoms in childhood	geometric mean 0.037 mg/m^3; subjects categorized into low and high formaldehyde using LOD; also sampled MCA, ICA and dust	(reduced recall accuracy? and potential for recall bias)	casting), comparable for age; smoking prevalence, prevalence female, and work duration higher in referent. Symptom prevalence compared between groups. Co-exposures measured but not adjusted for in analysis. Independent effect of formaldehyde could not be determined	adjustment for other irritants (isocyanic acid, methyl isocyanate, dust) which were strongly associated with symptoms. Also restricted analyses excluding asthma or allergies, females, or smokerswith similar results	N=69 of 84 referents	 Could not distinguish effect of formaldehyde from those of other irritants that were strongly associated with symptoms; Potential for information bias (reduced recall accuracy); potential health worker survival
Neghab et al. (2011) (prevalence)	100% participation; healthy worker survival?	Area samples (40 minutes, N=7) in 7 workshops and 1 in office area. Mean 0.96 mg/m^3; SD 0.49 mg/m^3; adequate exposure contrast for comparison of exposed and referent	Self-report, interview & American Thoracic Society questionnaire; symptoms at work	Referent from the same industry and comparable for socioeconomic status, age, smoking prevalence (25%). Symptom prevalence compared between groups.	Symptom prevalence compared by exposure group, chi-square	N=70 exposed N=24 referents	 Healthy survivor bias

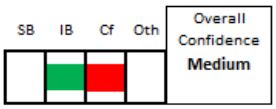
1 ***Supporting Material for Hazard Analyses of Sensory Irritation***

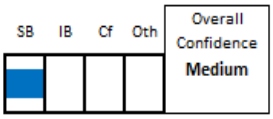
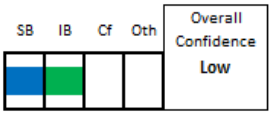
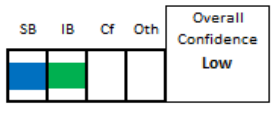
Table A-39. Summary of epidemiology studies of laboratory exposures to formaldehyde and human sensory irritation

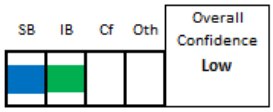
(Reference), study design, exposure levels	Results
Kriebel et al. (1993) (Massachusetts) Panel study, 24 clinical anatomy students dissecting cadavers during 10-wk lab once a wk, 3 hrs. **Outcome:** Symptoms recorded before, during and after the lab; ATS questionnaire for baseline and modified brief questionnaire during lab, references provided. **Exposure:** Personal samples in breathing zone (1- to 1.5-hr duration). Geometric mean 0.73 ppm (SD 1.22 ppm). Range 0.49−0.93 ppm (n=8). No trend in concentrations over semester. Formaldehyde levels in three air samples in the cavities of the cadavers were 3.0, 3.6 and 4.3 ppm. **Analysis:** Multivariate linear regression models; mean prelab and cross-lab change in symptoms analyzed using random effects models. 	Average symptom prevalence increased from beginning to end of weekly lab session by 43%. **Prevalence (%) Before, Midway and After Lab Session** **Symptom** / **pre** / **mid-** / **Post** Eyes 16 66 59 Nose 46 75 67 Throat 25 45 40 Breathing 16 41 36 Cough 15 26 20 Analysis indicated that magnitude of increase in symptom prevalence across lab session decreased as semester advanced (In week: eye ß -0.74, p = 0.002; throat ß -0.39, p = 0.03; nose ß -0.64, p = 0.06). No trend in prelab symptom severity over 10-week course
Uba et al. (1989) (California) Panel study, 1984-1985. 103 of 142 medical students in a 7-mo anatomy class, meeting twice a wk for 4 hrs (September 1984−April 1985), mean age (range): 24.3 (21–33) yrs. **Outcome:** Persistent symptoms: 103 students completed respiratory questionnaire (ATS) at the beginning (September 1984) and end of course (April 1985). Acute symptoms: 81/103 students completed different questionnaire after anatomy lab with formaldehyde exposure and after microanatomy lab (no formaldehyde) during Nov 1984. Order of questionnaires varied. **Exposure:** Personal samplers (impingers) in the breathing zone. TWA formaldehyde concentrations (N = 32 samples during different class periods over 7 months). Short-term samples (N = 16) for peak concentrations during dissection and observation. Dissecting room ventilated 24 hrs/d TWA concentrations: range, ≤ 0.05 (LOD) to 0.93 ppm (< 0.06 to 1.1 mg/m^3). During dissection: mean 1.9 ppm (2.3 mg/m^3); range 0.1 to 5.0 ppm (0.12 to 6.1 mg/m^3).	**Symptoms during lab session: symptom prevalence in anatomy lab (exposed) compared with microanatomy lab (unexposed) (N = 81)** Symptom / Ex-posed / Unexposed / Odds Ratio Itchy eyes 33 1 33* Watery eyes 36 3 12* Burning eyes 47 0 infinite Burning nose 19 0 infinite Sore throat 21 4 5.3** Sneezing 10 1 10** Rhinorrhea 13 3 4.3** Chest tightness 4 0 infinite Cough 5 4 1.3 Wheezing 2 0 infinite

(Reference), study design, exposure levels	Results
When observing dissection: mean 1.2 ppm (1.5 mg/m^3); range 0.2 to 2.0 ppm (0.25 to 2.5 mg/m^3). Monthly average in September, October, and May: 0.6, 0.8, 0.1 ppm (0.74, 0.98, and 0.12 mg/m^3). **Analysis:** Symptom prevalence at beginning of course compared to end of course, paired analysis, McNemar's test; symptom prevalence after lab with formaldehyde compared to lab with no formaldehyde, odds ratios, McNemar's test paired samples  .	Dyspnea 2 0 infinite McNemar's test paired samples, * p<0.001; **p<0.05 **Persistent symptoms (Number reporting symptoms only in September 1984 or only in April 1985)** Symptom / Sept. 1984 / April 1985 / Odds Ratio Cough 1 8 8.0* Phlegm 4 9 2.3 Chronic bronchitis 4 2 0.5 Chest illnesses 9 0 0** Wheezing 37 1 0.03** Wheezing with Dyspnea 4 0 0*** Dyspnea on exertion 0 0 - McNemar's test paired samples, * p = 0.02; ** p <0.001; ***p = 0.05
Mori et al. (2016) (Japan) Cross-sectional study, Students (2nd year), n=123 (98.4%) enrolled in afternoon gross anatomy classes, April–July 2013, mean age 22.9 yrs; compared to nonexposed 1st year students, n=114 (91.9%), mean age 21.2 yrs. 75% males **Outcome:** Questionnaire, 16 subjective symptoms, frequency never, sometimes, or often; administered April 2013 before, May 2013 during, and January 2014 6 mo after completion of course. **Exposure:** Area samples at breathing height, 5 locations during class in May 2013 on same day questionnaires were completed. Mean (SD) 0.123 (0.025) mg/m^3 (conversion by EPA). Area sample, 5 locations during class on same day questionnaires were completed. Mean (SD) 0.1 (0.02) ppm **Analysis:** Regression of presence or absense of symptoms in relation to exposure group on day of survey, controlling for doctor-diagnosed allergies, sex and age 	**Symptoms reported comparing exposed to unexposed on a day during gross anatomy class (OR (95% CI))** **Symptom / OR / 95% CI** Eye soreness 2.35 1.3–4.27 Eye strain 1.82 1.07–3.14 Itchy eye 0.75 0.43–1.31 Dry eye 1.11 0.63–1.96 Tearing 2.62 1.36–5.04 Itchy nose 1.76 1.01–3.06 Nasal 0.78 0.44–1.36 Runny nose 0.82 0.47–1.44 Sore throat 1.45 0.82–2.55 Dry throat 0.87 0.49–1.57
Kriebel et al. (2001) (Massachusetts)	Mean postlab intensity of eye, nose, and throat irritation decreased over semester.

(Reference), study design, exposure levels	Results
Panel study, 38 anatomy students (of 54 total) during 12-wk class meeting once per week, 2.5 hrs. Mean age 24.9 yrs, 23.7% male, 2 current smokers, 5 ex-smokers, 4 history of asthma **Outcome:** Symptom questionnaires before and after each lab session. Scale of symptom intensity ranged from 0 (not at all) to 10 (very, very much). **Exposure:** Continuous monitoring in 6 homogenous locations (LOD = 0.05 ppm [0.06 mg/m^3). 12-min work-zone concentrations for each student calculated using sampling data and recorded work locations. Geometric mean concentration over all lab sessions and participants: 0.7 ppm [0.9 mg/m^3] (GSD 2.13) Peak 12 min concentration 10.91 ppm (13.42 mg/m^3) Average ± SD concentration over all weeks and participants: 1.1 ± 0.56 ppm (1.4 ± 0.69 mg/m^3) Concentrations decreased over 12-wk semester. **Analysis:** Generalized estimating equation regression model accounting for lack of independence of repeated measures in individuals.  Attendance declined from n=37 to n=10 over 13 wks (better attendance by healthy individuals?)	**Association of symptom intensity with exposure during lab & interaction with time (Percent change in intensity per ppm or ppm-weeks)** | | Recent exposure[b] | Recent exposure x ln(week)[c] | |---|---|---| | Eye Irritation | 1.22* | −0.35* | | Nose Irritation | 1.09* | −0.42* | | Throat Irritation | 0.81* | −0.36* | *p <0.001 for significant deviation from slope = 0 [b]Mean concentration during 2.5-hr lab [c] Interaction between recent exposure and natural log of week number, indicating declining strength of association with time.
Takahashi et al. (2007) (Japan) Panel study, 2002–2003. 143 medical students (68.5% male, 88.8% 20–24 yrs of age) who dissected cadavers 15 hours per week for 2 mos and 76 students who had taken same course 2 to 4 years earlier (68.4% male, 77.6% 20–24 yrs of age). **Outcome:** Symptom questionnaire administered after 1st day of exposure and at end of course. **Exposure:** Area formaldehyde samples (> 10 min, 8 locations in room), upon opening of thorax, mean 2.12 ppm (SD 0.23), range 1.7−2.44 ppm (2.6 ± 0.28 mg/m^3, range 2.13−3.05 mg/m^3). Breathing zone samples (18/143 students), mean 2.4 ppm (SD 0.49), range 1.79−3.78 ppm; (mean 3.0 ± 0.61 mg/m^3, range 2.24−4.72 mg/m^3) **Analysis:** Prevalence after first exposure and at end of course compared, McNemar's test  Large gap between symptom ascertainment and exposure measurements	Prevalence after first exposure and at end of course estimated from Figure 1 in the paper. Largest increase in symptoms (p<0.05) reported for eye soreness (from about 35% to about 68% on 1st day versus end of course), lacrimation (12% to 60%), throat irritation (14% to 42%), eye fatigue (28% to 44%), rhinorrhea (17% to 35%), skin irritation (14% to 28%).

(Reference), study design, exposure levels	Results
Takigawa et al. (2005) (Japan) Intervention study, purpose: Evaluate installation of a ventilation system between phases and effects on formaldehyde concentrations and symptoms. 2 phases; 1st phase: 78 volunteer anatomy students in 2001 (mean age 21.6 yrs); 2nd phase: 79 volunteer anatomy students 3 yrs later in 2004 (mean age 21.7 yrs). **Outcome:** Self-administered questionnaires on health complaints before and during each 2-mo course. Symptom frequency: 1 (never), 2 (scarcely), 3 (sometimes), and 4 (always). Symptom change index: Symptom frequency score during session subtracted from score before course. **Exposure:** Area formaldehyde samples (>10 min, 9 locations in room); upon opening of thorax (represents highest concentration over 2 mos). Phase I: Median (range) 2.59 (2.1–3.0) mg/m^3 (concentration reported as 0.259 mg/m^3 in Table 3 of the paper must be an error). Phase II: Median (range) 0.729 (0.291–0.971) mg/m^3 Personal samples (measured with gas sampler on 24 students in first phase (42–962 min) and 46 in second phase (100−540 min)): Phase I: Median (range) 3.313 (2.238−8.909) mg/m^3 Phase II: Median (range) 0.878 (0.396−3.386) mg/m^3 **Analysis:** Symptom change index, 1st and 2nd phases compared; Mann-Whitney test, p <0.05.  Large gap between symptom ascertainment and exposure measurements	Symptom change indexes for 8 of 25 measured symptoms were significantly less comparing the second phase results with the first phase results. **Symptom Change Index** |	Symptom	1st (N=78)	2nd (N=79) | |---|---|---|---| | Skin	Eczema	0.13	-0.09 | | Eye	Itchy	0.74	0.27 | |	Irritated	0.96	0.52 | |	Watery	1.42	0.46 | |	Poor vision	0.17	-0.27 | | Nose	Itchy	0.67	0.22 | |	Changed sense smell	0.18	0.33 | | Throat	Sore	0.69	0.22 |
Wantke et al. (2000) (Austria) Panel study, 27 medical students, participants in Wantke et al. (1996) enrolled in a 2nd dissection class, 55.6% male **Outcome:** Symptoms standardized questionnaire at beginning, in middle, and at end of 10-wk course. Daily symptom cards during class **Exposure:** Continuous measurements for formaldehyde and phenol at 2 locations during lab, exposures for 43 d Formaldehyde Mean 0.265 ± 0.07 mg/m^3, range 0.133−0.410 mg/m^3, Phenol Mean 4.65 ± 2.96 mg/m^3, range 0.09−11.8 mg/m^3 **Analysis:** Prevalence in November and December compared to October, McNemar exact test	Symptom prevalence was not correlated with smoking, or type I allergy, complaints of dizziness occurred only in males **Prevalence of Symptoms at Beginning, Middle (5 Wks) and End (10 Wks) of Course** | Symptoms	Before	Middle	End | |---	---	---	---| | Burning eyes	0.111	0.481**	0.333* | | Sneezing	0.074	0.037	0.037 | | Nosebleed	0.185	0.111	0.185 | | Cough	0.074	0.148	0.074 |

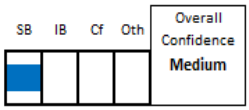
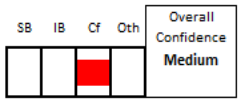
(Reference), study design, exposure levels	Results
 See Wantke et al. (1996b)	Shortness of breath 0 0.185 0.037 *p <0.05, **p <0.01
Wantke et al. (1996b) (Austria) Panel study, 1995. 45 medical students enrolled in 1st dissection class, 51.1% male, age 20.9 yrs, 3 hr sessions, 5 d/wk for 4 wks **Outcome:** Symptoms, standardized questionnaire at beginning and at end of 4-wk course **Exposure:** Continuous measurements for formaldehyde, 2 locations during lab; Mean 0.124 ± 0.05 ppm, range 0.059−0.219 ppm No sampling for phenol **Analysis:** Compared symptom prevalence during course to before, McNemar exact test  Low participation, possibility of selection bias away from null; Potential recall issues − symptoms for previous weeks	**Prevalence of Symptoms During 4 Wk Course** Symptoms: Before / During / p-Value Burning eyes: 0.133 / 0.289 / < 0.02 Sneezing: 0.244 / 0.089 / NS Nosebleeds: 0.244 / 0.044 / NS Cough: 0.044 / 0 / NS Shortness of breath: 0 / 0.022 / NS Symptom prevalence was not correlated with gender, smoking, or type I allergy.
Chia et al. (1992) (Singapore) Cross-sectional study. 1st year medical students in anatomy lab, 150 of 164 total (91.5%); referent 189 3rd and 4th yr medical students, no recent formaldehyde exposure; matched on age, sex, and ethnicity. **Outcome:** Symptoms during previous 4 wks of anatomy course (twice per wk, 2.5 hr (or other activities for referent), assessed via a modified MRC standardized questionnaire **Exposure:** Area samples at dissecting tables, n=6, collected on two occasions, Mean (SD) 0.5 ppm (0.08), range 0.4−0.6 ppm Personal samples, n=14 students, duration 2.5 hrs, Mean (SD) 0.74 (0.18), range 0.41−1.2 ppm LOD 0.05 ppm **Analysis:** Symptom prevalence in exposed compared to referent  Questions about dissimilarity of 1st and 4th year students and potential for recall bias during previous 4 weeks of course	**Prevalence of Symptoms** Symptom: Exposed (n = 150) / Referent (n = 189) / p-Value Decreased ability to smell: 0.127 / 0.032 / 0.002 Eye irritation: 0.8 / 0.132 / < 0.001 Throat irritation: 0.313 / 0.138 / < 0.001 Dry mouth: 0.18 / 0.058 / < 0.001 No statistically significant difference for other symptoms (cough with mucus, chest tightness, chest pain, and breathlessness) (data were not reported).
Fleisher (1987) (New York) Cross-sectional study 1st year medical students (N = 89) (43.6% of total 204 surveyed) (71% male) in gross anatomy course (formaldehyde	Symptoms prevalence (% reporting symptom all or some of the time) among 38 students responding to both questionnaires (N=38)

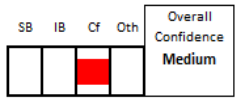
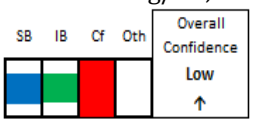
(Reference), study design, exposure levels	Results
exposed). Referent: Same students (n=60) (72% male) in pathology/microbiology laboratory six months later. 98.9% of all students attended 75−100% of all lab sessions. **Outcome:** Symptoms questionnaire one month after end of course. Symptom frequency: all of the time, some of the time, rarely or never. **Exposure:** Area formaldehyde measurements in 6 anatomy labs, one day during semester, 1983; sampling time 188−222 minutes. Personal breathing zone samples (3M Diffusion), 2 instructors, sampling time 180−190 min Area samples: Drager tubes (all labs): <LOD (1 ppm) NIOSH method (3 labs): LOD (0.02 ppm), 0.03, 0.59 ppm; Breathing zone: 0.18 and 0.69 ppm; **Analysis:** Within person comparisons; *t*-test comparing mean symptom scores  Low response to both questionnaires and selection potential; temporal gap in symptom response reduced recall accuracy potential	**Symptom** / **Anatomy** / **Path/ Micro** Eye Irritation 68.4* 21.0 Nose Irritation 61.1* 13.1 Sneezing 37.8* 15.8 Tightness in chest 11.1 2.6 Shortness of breath 8.3* 0.0 Cough 28.6* 5.3 Throat Irritation 38.9* 7.9 Sinus problems 35.1* 5.3 *$p < 0.05$

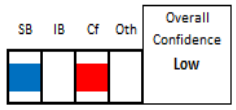
GSD = geometric standard deviation; MRC = Medical Research Council; NIOSH = National Institute of Occupational Safety and Health; ND = not detected.

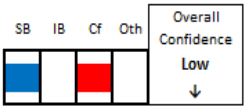
Table A-40. Summary of epidemiology studies of occupational exposures to formaldehyde and human sensory irritation

(Reference), study design, exposure levels	Results
Neghab et al. (2011) (Iran) Prevalence survey, 70 male exposed workers with ≥2-year history of exposure at a melamine-formaldehyde resin producing plant (mean (SD) age: 38.2 (8.4) years; mean (SD) work duration 13.2 (7.8) yrs. 24 male, healthy referent employees with no current or history of exposure to formaldehyde or other respiratory toxicants (mean (SD) age: 40.0 (8.2) yrs); mean (SD) work duration 14.5 (8.1) yrs. 100% participation. **Outcome:** Respiratory symptoms ascertained via interview using standardized questionnaire (ATS). **Exposure:** Area samples (40-minute sampling time) in 7 workshops (N=7) and offices (N=1) Formaldehyde concentration: ppm, mean (SD): Exposed: 0.78 (0.40) (0.96 (0.49) mg/m^3) Referent: nondetectable **Analysis:** Symptom prevalence compared	**Prevalence Respiratory Symptoms:** Symptom / Exposed / Referent Cough 20%* 0% Phlegm 28.6%* 0% Chest tightness 52.9%* 0% *$p < 0.05$

(Reference), study design, exposure levels	Results
 Concern for healthy worker survivor bias	
Holness and Nethercott (1989) (Toronto, Canada) Prevalence survey, 84 of 97 selected funeral service apprentice workers from funeral homes selected by the Metropolitan District Funeral Director's Association (mean (SD) age 32.1 (11.1) yrs, 89% male, work duration 8.2 yrs (SD 9.9)). 38 service volunteers and paid student volunteers as referent subjects similar in age to the apprentices (mean (SD) age 28.7 (12.7) yrs, 84% male, work duration 7.2 yrs (SD 11.9)). **Outcome:** Questionnaires (ATS) administered before and after an embalming procedure. **Exposure:** Area samples (N=2) during each embalming procedure, mean sampling duration (range): 85 minutes (30–180 minutes). Mean (SD) formaldehyde: Exposed: 0.36 (0.19) ppm (0.44 (0.23) mg/m^3)[a], range 0.08–0.81 ppm. Autopsied cases 0.44 ppm. Average levels were 0.21 ppm when ventilation units were in operation. Referent: 0.02 ppm (0.025 mg/m^3)[a] **Analysis:** Differences evaluated using logistic regression analysis controlling for smoking (pack-years).  Groups selected from different source populations	Prevalence elevated for 12 of 13 eye, URT, respiratory and cutaneous symptoms, but 5 were significantly higher compared with referent: chronic bronchitis (20% vs. 3%, p = 0.035), shortness of breath (20% vs. 3%, p = 0.043), nasal (44% vs. 16%, p = 0.003) and eye (42% vs. 21%, p = 0.026) irritation and past skin problem (42% vs. 13%, p = 0.003).
Horvath et al. (1988) (Wisconsin) Prevalence survey, 109 of 159 workers at a particleboard manufacturing plant (71% participation); 57% male; mean age 37.4, SD 11.7 years; Mean duration of employment 10.3 years (1 – 20 years); Referent: 254 of 300 workers at 2 food plants (44% male; mean age (SD): 34.2 (10.6) years. **Outcome:** Respiratory symptoms questionnaire (American Thoracic Society, ATS) completed before and after monitored work shift. Intensity assessed by subjects with visual analog scale. **Exposure:** Personal and area samples; 8-hr, TWA concentrations measured on each worker on the day of examination. In the particleboard plant, TWA values averaged 1.04 mg/m^3; range 0.26 to 4.4 mg/m^3. In the food plants, TWA values averaged 0.08 mg/m^3, range 0.03 ppm to 0.12 ppm). Other agents sampled in particleboard or molded products plant.	**Symptom Prevalence While at Work Reported in Preshift Questionnaire:** Symptom — Exposed — Referent Nose/ throat irritation — 43.9%* — 13.0% Eye irritation — 49.5%* — 24.0% *p < 0.05 **Symptom Prevalence Reported at End of Shift:** Symptom — Exposed — Referent Throat sore/burning — 22.0%* — 3.9% Cough — 34.9%* — 18.9% Phlegm — 26.6%* — 9.8% Nose burning — 28.4%* — 2.0% Stuffy nose — 33.9%* — 14.2%

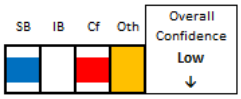
(Reference), study design, exposure levels	Results
Compound / Mean (Range) Total particulates[a] 0.38 (0.25-4.4) mg/m^3 Respirable particulates 0.11 (0.025-1.06) mg/m^3 Phenol 0.15 (0.11-0.26) ppm Carbon monoxide 7.35 (3.0-11.0) ppm Sodium hydroxide 0.4 – 0.21 mg/m^3 Nitrogen dioxide ND [a]Total particulates in food plants were 0.5 and 0.42 mg/m^3. **Analysis:** Prevalence compared using chi-square statistic. Dose-response of end of shift symptoms evaluated using multiple regression models. 	Itching nose 21.1%* 7.9% Eyes burning 39.5%* 9.1% Eyes itching 19.3%* 7.1% *p <0.05 Intensity (visual analogue scale, 0 – 100) for burning eyes, mean (SD) 47 (27) Shortness of breath (8.3 vs. 5.1%), wheezing (3.7 vs. 2.8%), and difficulty breathing (6.4 vs. 2.0%) were not significantly increased. Dose-response: formaldehyde a significant predictor of cough, chest complaints, phlegm, burning nose, stuffy nose, burning eyes, itchy nose, sore throat, and itchy eyes in multiple regression models; coefficients were not reported.
Löfstedt et al. (2011) Prevalence survey. Sweden 3 brass foundries producing cores using Hot Box method. 43 of 48 exposed workers; 69 of 84 referents working outside core-production and die-casting halls; not exposed to chemicals. Prevalence of "ever" asthma or childhood allergy lower in exposed than in referent (9% and 19%, respectively versus 14% and 35%, respectively, p<0.05) **Outcome:** Self-report, questionnaire; existence of symptoms during prior week; nasal signs **Exposure:** Individual measurements. Monoisocyanates: Mean of 4–5 5-min samples randomly distributed over entire shift. Formaldehyde: sampling over entire 8-hr shift Categorized low and high using LOD as cut-point (LOD not reported). Mean 0.51 mg/m^3, SD 0.049 mg/m^3, range 0.013–0.19 mg/m^3  Could not distinguish effect of formaldehyde from those of other irritants that were strongly associated with symptoms; Potential for information bias (reduced recall accuracy); potential health worker survival	**Associations of ocular and nasal symptoms within the previous week and nasal signs with formaldehyde exposure** **Any nasal symptoms** Referent (n=68) 1.0; Low (n = 30) 4.3 (1.7–11.2); High (n = 12) 4.7 (1.2–19.1) **Nasal signs – dry mucosa** Referent 1.0; Low 2.8 (1.1–6.9); High 2.8 (0.8–10.2) **Irritated eyes** Referent 1.0; Low NR*; High 6.3 (1.4–28.4) NR: not reported Nasal symptoms included discharge, itch, sneezing and congestion ICA and MIC also associated with these nasal endpoints, nasal symptoms OR 3.9 low and 5.0 in high exposed; nasal signs OR 4.5 low and 1.9 high exposed
Alexandersson and Hedenstierna (1989); Alexandersson et al. (1982) (Sweden) Prevalence survey, 1980, Employees at carpentry works (N=47) for > 1 yr, regularly exposed to formaldehyde, and working on the study day, mean age (± SE) 35 (1.8) yrs, 49% smokers, duration employment 5.9 years. Referent (N=20) not exposed	**Symptom Prevalence at Work, 1980 (%)** Eye: Exposed 74; Referent 0 Nose, Throat: Exposed 36; Referent 0

(Reference), study design, exposure levels	Results
to formaldehyde or other lung irritants, employed at the same plant, mean age (± SE) 35.3 (2.3) years. Asthmatics excluded. Follow-up 5 yrs later (1984), 34 exposed and 18 referents; 21 remained exposed, 13 transferred to tasks with no exposure to irritants. **Outcome:** Interviews using standardized questionnaire focused on nose, eyes, upper airways, and lungs, chronic bronchitis defined by British Medical Research Council. **Exposure:** 1980 study: Personal samplers for formaldehyde, terpenes, and dust, N=31, duration 6–7 hr/d; Mean concentration (range): formaldehyde 0.47 mg/m^3, 0.05–1.62 mg/m^3, terpenes 0 (0–9) mg/m^3, dust 0.5 (0.3–0.7) mg/m^3 1984 study: 3–4 15 min samples per person in the exposed group, estimated TWA Mean TWA concentration (± SD): formaldehyde 0.50 (0.12) mg/m^3 Mean Peak concentration (± SD): formaldehyde 0.69 ± 0.68 ppm **Analysis:** Prevalence of symptoms while at work, change from 1980 to 1984, chi-square  Healthy survivor bias	**Symptom Prevalence at Work, 1984 (%)** Ex-posed / Trans-ferred / Referent **Eyes** Smarting: 45 / 30 / 0 Itching: 40 / 20 / 17 Running: 60 / 30 / 12 **Nose** Running: 30 / 10 / 12 Dryness: 15 / 0 / 6 ↓ Smell: 0 / 0 / 0 Change from 1980 to 1984 not statistically significant, p >0.05
Herbert et al. (1994) Prevalence survey, 99 oriented strand board (OSB) workers (exposed, 98% participation), mean age 35.4 yrs, 51.5% smokers; work duration 5.1 yrs; 165 oil/gas field plant workers (not exposed to formaldehyde or oil and gas vapors) from same geographic area (82% participation), mean age 34.9 yrs, 27.9% smokers, work duration 10 yrs. Excluded 14 workers in referent with hydrogen sulfide exposure. **Outcome:** Respiratory symptoms ascertained via interview using standardized questionnaire. **Exposure:** Time weighted average formaldehyde and dust concentrations based on 21-hr continuous sampling in the breathing zone at 5 work sites on 2 separate days. Formaldehyde: range 0.07–0.27 ppm (0.09–0.33 mg/m^3). Dust mean: 0.27 mg/m^3, 2.5 µm diameter **Analysis:** Symptom prevalence compared  Different prevalence smoking and duration of employment between exposed and referent; no adjustment in analyses	**Prevalence Respiratory Symptoms (relevant to URT irritation):** Symptom / Exposed / Referent Usual Cough: 24.5%* / 11.1% Usual Phlegm: 31.3%* / 13.3% Chest tightness: 43.4%* / 22.8% *p < 0.05
Holmström et al. (1991)	**Rate Difference (%) in Symptoms, Exposed versus Referent**

(Reference), study design, exposure levels	Results
Sweden Prevalence survey, Group 1: 16 persons exposed to medium density fiberboard (MDF) dust for at least 30% of the workday, mean age 44.1 yrs, 100% male, 38% smokers. Group 2: 29 exposed to other types of wood dust, mean age 39.3 yrs, 86.2% male, 31% smokers. Group 3 (Referent), 36 governmental clerks living in same village as chemical plant, mean age 39.9 yrs, 47.2% male, 28% smokers. (Groups 2 and 3 same as for (Holmström and Wilhelmsson, 1988) **Outcome:** Symptom prevalence; Questionnaire and medical examination **Exposure:** Personal exposure measurements stable through year, average 0.2–0.3 mg/m^3, peaks seldom > 0.5 mg/m^3, Formaldehyde Concentration, mean MDF 0.26 mg/m^3, range 0.17–0.48 mg/m^3 Wood dust 0.25 mg/m^3, range 0.3–1.0 mg/m^3 Referent 0.09 mg/m^3 **Analysis:** Exposed compared to referent; prevalence rate difference, 95% confidence intervals  Healthy survivor bias; groups selected from different source populations; Potential confounding and no adjustment in analyses	Symptom / MDF / Wood Dust % / 95% CI / % / 95% CI Nasal 66 47, 85 3 −20, 26 Eye 38 13, 64 1 −1, 13 Throat 19 -3, 42 4 −8, 18 Lower airway 36 9, 63 3 −14, 21 Relief from symptoms during weekends in 80% in MDF group and 67% in wood dust group; and during vacations.
Alexandersson and Hedenstierna (1988) (Sweden) Prevalence survey, 38 exposed employees working with acid-hardening lacquers for the previous 12 mos (mean age (SD): 34 (10) yrs, mean duration employment 7.8 yrs) and at work on the study day. 18 referent employees at the same company (mean age (SD): 37 (9) yrs). Asthmatics excluded. **Outcome:** Interviews regarding irritation of eyes, nose, throat, lungs and bronchi were conducted using a standardized questionnaire. **Exposure:** Formaldehyde measurements in the breathing zone, 3–4 15 min samples per person in the exposed group. No formaldehyde measurements reported for referent group. Formaldehyde TWA: 0.40 mg/m^3, range: 0.12–1.32 mg/m^3. Peak concentration (15 min): 0.70 mg/m^3, range: 0.14–2.6 mg/m^3. Additional measurements of solvents and dust (4 hr) **Analysis:** Group comparisons, chi-square statistic  Selection for healthy survivors; Potential confounding and no adjustment in analyses	**Symptom Prevalence at Work** Exposed N (%) / Referent N (%) Eye 25 (65.8) 3 (16.7) Nose, Throat 15 (39.5) 0 Dyspnea 4 (10.5) 0 Chest oppression 4 (10.5) 0 Cough 2 (5.3) 0

<table>
<tr><th>(Reference), study design, exposure levels</th><th>Results</th></tr>
<tr><td>
Wilhelmsson and Holmstrom (1992); Holmström and Wilhelmsson (1988) (Sweden)

Prevalence survey, three test groups chosen by the Swedish Board of Occ. Safety and Health. Group 1: 70 exposed to formaldehyde at a chemical plant (resins and impregnation of paper for laminate production), mean age 36.9 yrs, 87% male, work duration 10.4 yr (SD 7.3), range 1–36 yr. Group 2: 100 exposed to wood dust and formaldehyde, mean age 40.5 yrs, 93% male, work duration 16.6 yr (SD 11.3), range 1–45 yr. Group 3 (referent), 36 governmental clerks living in same village as chemical plant, mean age 39.9 yrs, 56% male, work duration 11.4 (SD 5.4), 4−18 yr.
Outcome: Questionnaire and medical examination, excluding upper airway infections. Atopics identified and analyzed separately from nonatopics based on a laboratory test utilizing the allergosorbent principle.
Exposure: Breathing zone (personal samplers, 1–2 hrs), mean, range 1985: Group 1: 0.26 (SD 0.17) mg/m^3; 0.05−0.50 mg/m^3. Group 2: 0.25 (SD 0.05) mg/m^3; 0.2−0.3 mg/m^3 and 1.65 mg/m^3 for wood dust.
Group 3 Referent: 0.09 mg/m^3
Cumulative exposure (dose-years) based on JEM
No occupational exposure to solvents; other agents (phenol, ammonia, epichlorhydrin, methanol, and ethanol) less than 1% above PEL.
Analysis: Compared symptom prevalence across exposure groups, chi-square

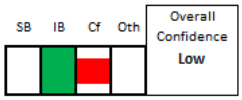


Healthy survivor bias; groups selected from different source populations; Potential confounding and no adjustment in analyses
</td><td>
Significantly increased symptom prevalence reported in formaldehyde exposed groups
<table>
<tr><th></th><th colspan="3">Exposure Group</th></tr>
<tr><th></th><th>1</th><th>2</th><th>3</th></tr>
<tr><td>Nasal</td><td>64%*</td><td>53%*</td><td>25%</td></tr>
<tr><td>Eye</td><td>24%*</td><td>21%</td><td>6%</td></tr>
<tr><td>Deep airway discomfort</td><td>44%*</td><td>39%*</td><td>14%</td></tr>
</table>
*$p < 0.05$

No significant difference between atopics vs. nonatopics in symptom prevalence.

Majority reported symptoms did not change over time
</td></tr>
<tr><td>
Kilburn et al. (1985a) (Los Angeles)
Prevalence survey, 76 female histology technicians in 23 hospitals & 2 labs (exposed), 97% of eligible, mean (SD) age 40.8 (11.6) yrs, work duration 12.8 (9.3) yrs; 56 women in referent (secretaries and clerks in same institutions) matched with 40 of the technicians for age, cigarette smoking, and ethnicity, mean (SD) age 39.5 (10.5) yrs.
Outcome: Questionnaire for symptoms; composite experience for previous months or years
Exposure: Environmental samples for formaldehyde, xylene, toluene, and chloroform by regional NIOSH laboratory in 10 of 25 labs; 1−4 hrs sampling time.
Collected information on exposures, work practices and ventilation.
Tissue specimen preparation,
</td><td>
Formaldehyde, xylene and toluene concentrations were not correlated with symptoms (data not shown).

Symptom Prevalence (%) by Duration of Formaldehyde Exposure (hours)
<table>
<tr><th></th><th></th><th colspan="3">Formaldehyde (Hours)</th><th colspan="2">>4 hours[1] Xylene: # Slides Cover slipped</th></tr>
<tr><th>Symptom</th><th>Ref</th><th>0</th><th>1−3</th><th>>4</th><th><100</th><th><100</th></tr>
<tr><td>Number</td><td></td><td>7</td><td>22</td><td>47</td><td>27</td><td>20</td></tr>
<tr><td>< odor[2]</td><td>5</td><td>14</td><td>32</td><td>32</td><td>22</td><td>45</td></tr>
<tr><td>Eye</td><td>20</td><td>28</td><td>59</td><td>66</td><td>63</td><td>70</td></tr>
<tr><td>Throat</td><td>12</td><td>14</td><td>36</td><td>49</td><td>37</td><td>65</td></tr>
</table>
</td></tr>
</table>

(Reference), study design, exposure levels	Results					
Formaldehyde 0.2–1.9 ppm (0.25–2.34 mg/m^3)[a]; rooms with tissue processors, xylene 8.9–12.6 ppm, chloroform 2–19.1 ppm; Staining and cover-slipping, xylene 3.2–102 ppm, toluene 8.9–12.6 ppm. Clerical offices Formaldehyde ND; xylene ND **Analysis:** Prevalence by hours formaldehyde exposure and xylene exposure (statistical analyses not provided).  Reduced recall accuracy over extended period	**Dry Mouth**	20	43	50	47	41	55
	Cough						
	Dry	9	14	23	34	22	50
	Mucous	9	14	0	19	7	35
	Blood	0	0	0	8.5	4	15
	Chest						
	Tight	5	14	27	40	26	60
	Pain	5	14	23	40	37	40
	[1] Xylene exposure among those with >4 hrs exposure to formaldehyde. [2] Decreased odor perception.						

CI = confidence interval; MDF = medium density fiberboard; OR = odds ratio; OSB = oriented strand board; SE = standard error.
[a]Concentrations reported by authors as ppm or ppb converted to mg/m^3

A.5.3. Pulmonary Function

Literature Search

A systematic evaluation of the literature database on studies examining the potential for effects on pulmonary function in relation to formaldehyde exposure was initially conducted in November 2012, with yearly updates to September 2016 (see Section A.5.1). A systematic evidence map identified literature published from 2016 to 2021 (see Appendix F). The search strings used in specific databases are shown in Table A-41. Additional search strategies included:

- Review of reference lists in the the articles identified through the full screening process and
- Review of reference lists in the 2010 draft Toxicological Review for Formaldehyde (U.S. EPA, 2010).

This review focused on standard quantitative measures of pulmonary function including spirometric measures, FEV_1, FVC, and FEF_{25-75}, as well as PEF measured using a flowmeter. Inclusion and exclusion criteria used in the screening step are described in Table A-42. The search and screening strategy, including exclusion categories applied and the number of articles excluded within each exclusion category, is summarized in Figure A-25. Based on this process, 53 studies were identified and evaluated for consideration in the Toxicological Review.

Table A-41. Summary of search terms for pulmonary function

Database, search parameters	Terms
PubMed No date restriction	(Formaldehyde[majr] OR paraformaldehyde[majr] OR formalin[majr]) AND ("pulmonary function" OR "lung function" OR "spirometr*")

Database, search parameters	Terms
Web of Science No date restriction	TS=(Formaldehyde OR paraformaldehyde OR formalin) AND TS=(pulmonary function OR lung function OR spirometry)

Abbreviations: Majr= major topic (filter); TS= the requested "topic" is included as a field tag

Table A-42. Inclusion and exclusion criteria for studies of pulmonary function

	Included	Excluded
Population	**Human**	**Animals**
Exposure	• Indoor exposure via inhalation to formaldehyde • Measurements of formaldehyde concentration in air, or exposure during dissection or embalming	• No formaldehyde specific analyses • Job title/industry-based analysis • Dermal • Outdoor exposure
Comparison	• Evaluated outcome associations with formaldehyde exposure	• Case reports • Surveillance analysis /Illness investigation (no comparison)
Outcome	• Reported measure of FVC, FEV, FEF or PEF based on spirometry or flowmeter	• Pulmonary function among asthmatic subjects in controlled human exposure studies (there were evaluated in the section on other respiratory conditions including asthma • Exposure studies/no outcome evaluated • Studies of other outcomes
Other		• Reviews and reports (not primary research), letters, meeting abstract, no abstract, methodology paper

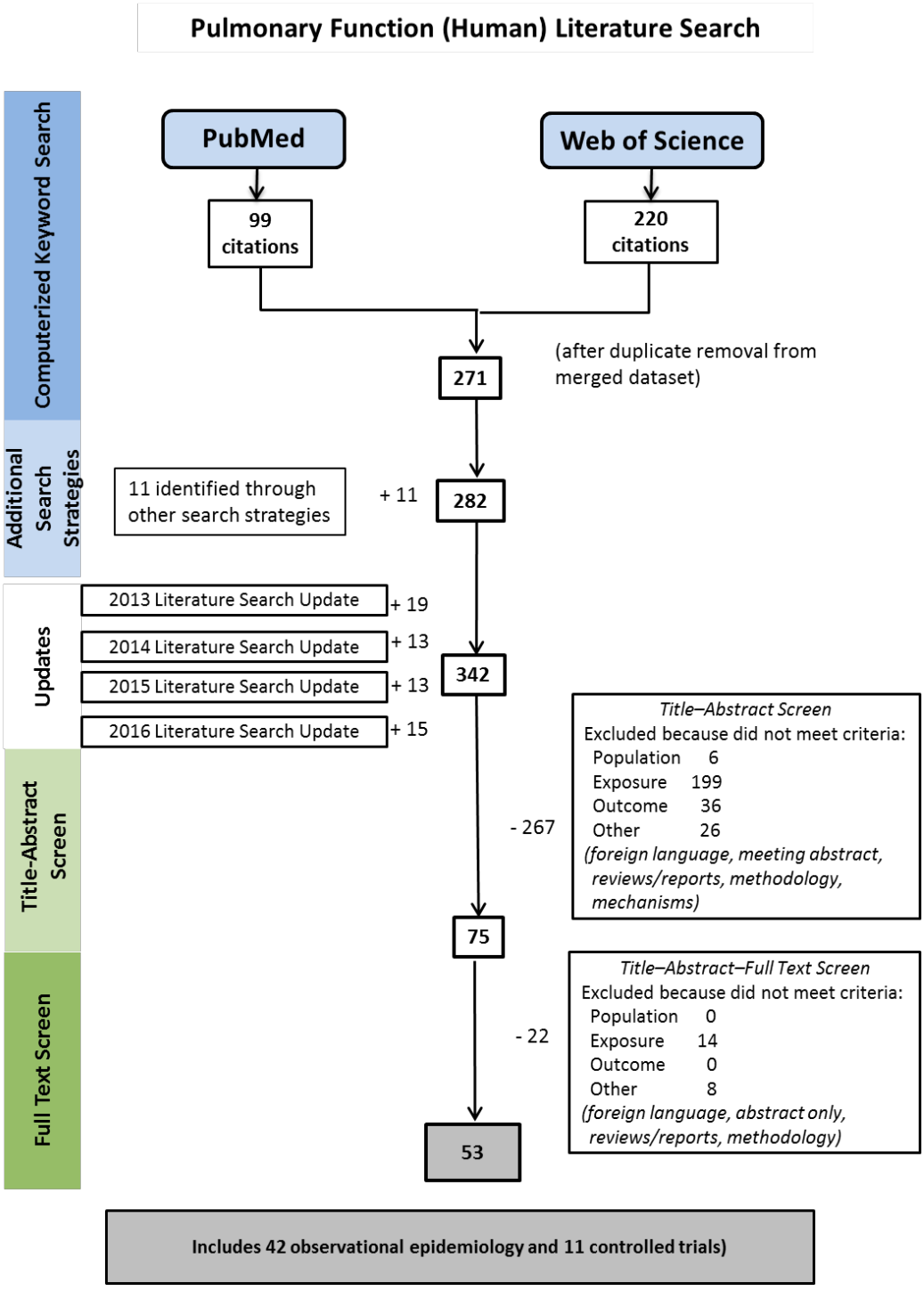


Figure A-23. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and pulmonary function in humans.

Study Evaluations

The American Thoracic Society has published guidelines for equipment performance requirements, validation, quality control, test procedures, and reference equations for each type of spirometric measurement (Miller et al., 2005a; Miller et al., 2005b), as well as the interpretation of testing results (Pellegrino et al., 2005). In addition to the use of conventional spirometric equipment, peak expiratory flow has been measured in research settings using portable flow meters operated by study participants trained in their use. Although it requires careful training and monitoring, this method has the advantage in that it can be used in large epidemiological studies and multiple measurements can be obtained over time. Studies of residential exposure to formaldehyde were conducted in this way (Krzyzanowski et al., 1990) .

Based on the evaluation of participant selection, exposure and outcome classification, confounding, and other limitations, a level of confidence in the study results, high, medium, low or not informative was assigned to each study. Eight studies with one or more critical limitations were classified as not informative.

Lung function varies by race or ethnic origin, gender, age, and height, and is best compared when normalized to the expected lung function based on these variables (Pellegrino et al., 2005; Hankinson et al., 1999). Analyses were considered to be limited if they did not adjust or otherwise account for these variables. Lung function also has been associated with smoking status and socioeconomic status (Chan-Yeung, 2000). These predictors of lung function were considered as potential confounders in the evaluation of studies of formaldehyde exposure. FEV_1 and PEFR exhibit diurnal variation, and this complicates the interpretation of changes across a work shift or during a laboratory session if no comparisons were made with an unexposed group (Chan-Yeung, 2000; Lebowitz et al., 1997). Studies with no comparison were given less weight in evaluating study results.

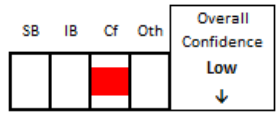
The healthy worker effect and survivor (lead time) bias was a concern for several cross-sectional occupational studies, some of which had no other major limitations. Removal of individuals more sensitive to the irritant effects of formaldehyde from jobs or tasks with formaldehyde exposure likely occurred in industries with high formaldehyde exposures, and this type of selection bias might result in an attenuation of risk estimates or a null finding if these individuals also experienced effects on pulmonary function.

Table A-43. Criteria for categorizing study confidence in epidemiology studies of pulmonary function

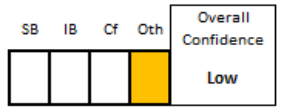
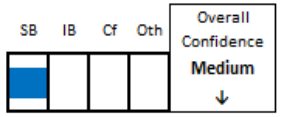
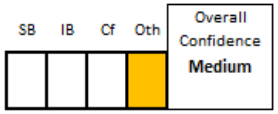
Confidence	Exposure	Study design and analysis
High	**General population:** For short-term exposure, sampling period coincides with pulmonary function measurements. For long-term exposure, exposure measure based on at least 3-d sample, corresponding to appropriate time window (e.g., measures in more than one season if time window	Population-based selection of participants or selection of workers at beginning of exposures (no lead time bias). Instrument for data collection described or reference provided (e.g., ATS guidelines) and outcome measurement conducted without knowledge of exposure status. Analytic approach evaluating dose-

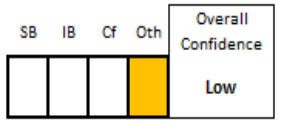
Confidence	Exposure	Study design and analysis
	covers 12 mos, or addressed season in the analysis). Exposure assessment designed to characterize mean individual exposures appropriate to analysis. **Work settings:** Ability to differentiate between exposed and unexposed, or between low and high exposure.	response relationship using analytic procedures that are suitable for the type of data, and quantitative results provided. Confounding considered and addressed in design or analysis; large sample size (number of cases).
Medium	**General population:** More limited exposure assessment, or uncertainty regarding correspondence between measured levels and levels in the etiologically relevant time window. **Work settings:** Referent group may be exposed to formaldehyde or to other exposures affecting respiratory conditions (potentially leading to attenuated risk estimates)	Lead time bias may be a limitation for occupational studies. Instrument for data collection described or reference provided, and outcome measurement conducted without knowledge of exposure status. Analytic approach more limited; confounding considered and addressed in design or analysis but some questions regarding degree of correlation between formaldehyde and other exposures may remain. Sample size may be a limitation.
Low	**General population:** Short (<1 d) exposure measurement period without discussion of protocol and quality control assessment. **Work settings:** Short sampling duration (<1 work shift) without description of protocol.	Lead time bias may be a limitation for occupational studies. High likelihood of confounding that prevents differentiation of effect of formaldehyde from effect of other exposure(s), limited data analysis (or analysis that is not appropriate for the data) or small sample size (number of cases).
Not informative	Exposure range does not allow meaningful analysis of risks above 0.010 mg/m^3; no information provided.	Description of methods too sparse to allow evaluation.

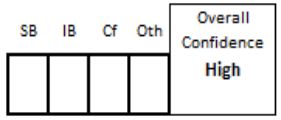
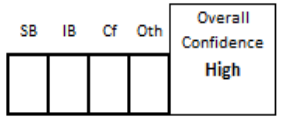
Table A-44. Evaluation of formaldehyde - pulmonary function epidemiology studies

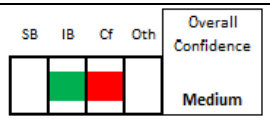
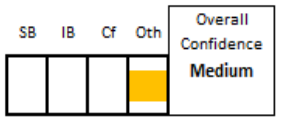
Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Laboratory Students Studies							
Akbar-Khanzadeh et al. (1994) (Cross-sectional)	Selection of participants not described. Medical students and instructors in anatomy lab; referents were nonmedical students and instructors.	TWA personal breathing zone samples obtained on all exposed subjects, 9 d, and 1 unexposed. 6 d Range 0.086–3.62 mg/m^3 Also sampled methanol (mean 110 ppm) and phenol (not detected)	Pre- and postlab spirometry using ATS criteria on 1 d per student; all had at least 6 wks of formaldehyde exposure at time of spirometry	Within person change across one lab. Age (26 vs. 32 yr), height and weight similar between exposed and unexposed; 21% with history of asthma in exposed and none in referent; nonsmokers	Mean (SD) absolute value at baseline and mean % difference across lab compared within and between groups; *t*-test	34 exposed; 12 referents	Cross-lab change  Reporting deficiencies; small sample size in referent
Akbar-Khanzadeh and Mlynek (1997) (Cross-sectional)	Selection of participants not described.	Personal (breathing zone) (n = 44) and area (n = 76) formaldehyde samples Range 0.34–5.47 mg/m^3	% predicted; prelab and postlab spirometric variables; four students assessed each time	Variables expressed as a percentage of reference values accounting for height, weight, age, sex, and race; all nonsmokers. *Since data collection occurred*	Mean cross-lab change analyzed within and between groups using regression model and *t*-test	50 exposed; 36 referents	Cross-lab change  Analyses did not account for possible acclimatization to formaldehyde over time.

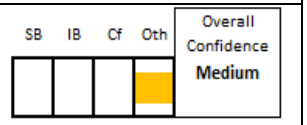
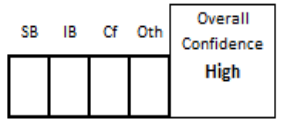
Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
				throughout the course, analyses did not account for acclimatization to formaldehyde over time.			
Binawara et al. (2010) (Cross-sectional)	Excluded individuals with symptoms, stress, type-1 allergy, respiratory disease, and smokers First-year medical students in anatomy lab	No formaldehyde measurements	Pre- and postlab spirometry, % predicted, day of course not reported	Within person change	Percent predicted prelab compared to postlab means (SD), *t*-test; no comparison group	N=80	Cross-lab change  No comparison group
Chia et al. (1992) (Cross-sectional)	Subjects selected randomly; all agreed to participate	Area samples at dissecting tables, *n* = 6, collected on two occasions. Personal samples, n=14 students, duration 2.5 hrs Range 0.50−1.48 mg/m^3	Spirometric measures (published methods); once before and after dissection, 1st d after 2-wk vacation.	Within person change; before and after dissection means adjusted for age and height, stratified by sex.	Means, absolute values adjusted for age and height, stratified by gender; and *p*-values; no SE; no comparison group	N=22	Cross-lab change  No comparison group; Small sample size

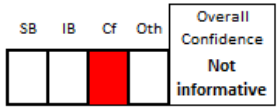
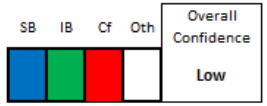
Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Khaliq and Tripathi (2009) (Cross-sectional)	Participants randomly selected; excluded students with respiratory illness or previous exposure to formalin; all nonsmokers	No formaldehyde measurements. Formaldehyde exposure assumed for dissection classes	Pre- and postlab spirometry; 3 tests using best value, measured on 1st day of exposure and 24 hrs after	Within person change	Mean absolute value (SD) compared pre- and postlab, *t*-test; no comparison group	N=20	Cross-lab change  No comparison group; Small sample size
Kriebel et al. (2001) (panel study)	94% participation; attendance declined from n=37 to n=10 over 13 wks (better attendance by healthy individuals?)	Work-exposure matrix from sampling in 6 work zones, multiple days, and reported time spent in each zone Average 1.35 mg/m^3, 10-min peak 13.42 mg/m^3	Spirometric measures (ATS methods) before and at end of 13 wks. PEF, prelab and across-lab change every weekly lab session	Within person change; multiple measurements; 2 smokers and 7 ex-smokers, PEF in smokers no different from nonsmokers	PEF as fraction of value before 1st lab session; Individual prelab and cross-lab change data analyzed together in relation to recent, average and cumulative formaldehyde in single generalized estimating equations model. GEE adjusted for cold on lab day. Cross-lab change: no comparison group	N=38 of 51 with pre- and postlab measures for ≥1 week	Longitudinal  Decline in attendance, association with symptoms unknown Cross-lab change  No comparison group
Kriebel et al. (1993)	96% participation	Personal samples in the breathing zone, 1–1.5 hrs of 3-	PEF repeated measures Wright flow meter;	Within person change; multiple measurements; one smoker	Mean absolute value (SD) prelab and cross-lab change in	N=20 in analysis out of 24	Longitudinal 

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
(panel study)		hour lab; multiple days Range 0.60−1.14 mg/m^3 Pentachloro-phenol measured but not detected.	measured 1−3 times during each weekly lab		pulmonary function analyzed in separate models using random effects models including asthma, asthma*week, eye and nose or throat symptoms. Provided data and results of statistical analyses; Also showed absolute value (SD) and cross-lab change (SD) at weeks 1 and 2 and 9 and 10		Small sample size Cross-lab change  No comparison group
Mohammad 'pour, 2011, 1518771@@author-year} (cross-sectional)	30 veterinary students, male and female, aged 18−20 yr, nonsmokers; selection of participants not described	No formaldehyde measurements **Inadequate**	Pre- and postlab spirometry	Within person change; nonsmokers, age comparable	Mean absolute value (SD) compared pre- and postlab, ANOVA; tested interaction between sexes and exposure	N=15 females; N=15 males	 Exposure levels uncertain and likely variable in this occupational group
Saowakon et al. (2015) (Tailand) Medical students and	Students and faculty in gross anatomy dissection labs; selection, recruitment, and	Personal samplers (n = 36 students, 4 instructors); area samples, all NIOSH-2016 method; 3-hr	Siblemed 120 protable spirometer, completed before start of dissection and after end of	Within person change; all nonsmokers	Average change over one 3-hr lab session in the exposed group (Within person change), paired *t*-test. Uncertainty	N=36 students; n=4 instructors	 No comparison group

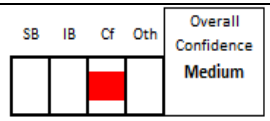
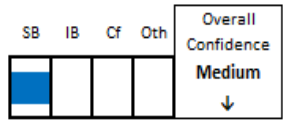
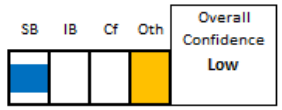
Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
academic staff	participation were not reported. Ages 19–21 yrs, nonsmokers with no history of chronic respiratory disease or symptomatic illness	samples over duration of class, 3 classes, January, August, and October Students: Mean (SD) ppm Class 1: 0.193 (0.120) Class 2: 0.271 (0.159) Class 3: 0.828 (0.182)	dissection lab, maximum of two readings		whether each participant was assessed more than once.		
Uba et al. (1989) (panel study)	72.5% participation	Personal sampling monitors (impingers) in the breathing zone; multiple days and during 3 different months TWA Range 0.06–1.14 mg/m^3	Spirometric measures (ATS methods); Absolute value (SD) pre- and postlab and cross-shift change before Day 0 (before exposure), at 2 wks and 7 mos	Within person change; all nonsmokers	Cross-shift change in pulmonary function analyzed using repeated measures ANOVA, adjusted for sex; change at 2 wks and 7 mos compared to the baseline day. Compared mean values measured at noon on baseline day, 2 wks and 7 mos.	N=96	Longitudinal  Cross-lab change 
Residential Studies and School Based Studies							
Bentayeb et al. (2015);	Elderly (20 randomly selected per	Measurements in common room; 1 wk	Assessed by same team in all countries;	Adjusted for sex, age, country, BMI, highest	General estimating equations analysis, accounting for	N = 600	Pulmonary function measures

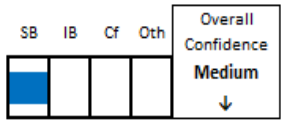
Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
(Cross-sectional), 2009–2011	home) permanently living in randomly selected nursing homes (8 per city) in selected city in 7 countries. Exclusion criteria stated (neurological or psychiatric disorders)	samples; also measured particulates, NO_2, ozone, temperature, humidity and CO_2; range of 1 wk averages 0.001–0.021 mg/m^3, median 0.006 mg/m^3; categorical (low and high) based on median concentration in each nursing home	medical visit and standardized questionnaire (European Community Respiratory Health Survey); spirometry (ATS/ European Respiratory Society guidelines), % predicted	school level, smoking, and season	correlations within nursing homes; adjusted OR (95% CI); stratification by presence or ventilation		 Confounding by co-exposures was not assessed; range of average concentrations within low and high exposure categories associated with overall effects is not known
Broder et al. (1988b, 1988c); Broder et al. (1988a) (Cross-sectional)	Identification of exposed through households with UFFI registered with state consumer agency; referents selected randomly from houses on adjacent streets; concern for possible over-	Area samples on 2 successive days in hallway, all bedrooms and yard. Median conc. in rooms were similar, Inside: referent 0.035 ppm, range 0.006–0.112 ppm [0.043 mg/m^3, range 0.007–0.138 mg/m^3]. 90% 0.061; UFFI	Spirometry protocol described	Adjustment for important confounders in data analysis	Regression models of spirometry values between and within each exposure group, analysis adjusted for total hrs spent in house/wk, outside temperature, gender, age, height, smoking, and race; presented only statistically significant	N=1,726 exposed; N=720 referent	 For within group analyses. Downgraded from high because results not presented for formaldehyde

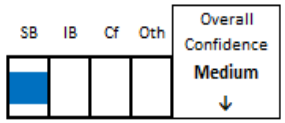
Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	reporting of symptoms but not for pulmonary function	0.043 ppm, range 0.007−0.227 [0.053 mg/m^3, range 0.009−0.279 mg/m^3], 90% 0.073 ppm Outside: referent 0.005 ppm, UFFI 0.005 ppm			regression coefficients; no data shown for formaldehyde associations		
Franklin et al. (2000) (Cross-sectional)	Recruitment through local schools; response rate of participants was not described. **Participation not expected to be influenced by outcome or exposure**	3−4 d passive samples in bedroom and main living area Median (IQR) 0.019 (0.011, 0.035) mg/m^3 (communication by author)	Spirometry protocol (ATS), measure-ments in clinic	Children with current or history of upper or lower respiratory tract disease were excluded. % predicted based on age, sex, and height. Mean eNOS levels by exposure category adjusted for age and atopic status	Mean absolute value (SD) and % predicted (SD) by exposure group (<50 and ≥50 ppb); only 10 homes in high exposure group (data provided by author); no demographic info except for age	N=224	 Limited exposure contrast**;** few subjects in high exposure group
Krzyzanowski et al. (1990), adults & children	A stratified random sample of 202 households of municipal employees; eligibility	Two one-week household samples, multiple locations Mean 0.032 mg/m^3;	PEF, Wright flow meter measured 4 times daily for 2 weeks	Potential confounding analyzed in analysis	Random effects model accounting for repeated measures, adjusted for asthma, acute respiratory illness,	N=202; repeated measures	

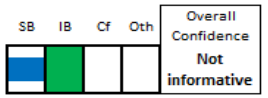
Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
(cross-sectional)	criteria described	maximum 0.172 mg/m^3			smoking, SES, NO_2, time of day; separate analyses for 15 yrs and younger, and over 15 yrs of age.		
Marks et al. (2010)	Schools and classrooms were selected using a 2-stage process, all students in selected classrooms (grades 4, 5, or 6) were recruited. Participation: 418 subjects (77%) of 543 students in selected classes.	One area sample in each classroom 2 d/wk for 6 wks	Spirometry protocol described	Randomized double blind intervention study of unflued and flued gas heaters, NO_2 and formaldehyde levels varied together in same direction	Analysis of effects in relation to heater use (flued vs unflued), correlated co-exposures	N=400	 No quantitative analyses specifically for formaldehyde
Norback et al. (1995) (Cross-sectional)	Recruited from 154 randomly selected members of general population; 57% participated. Possibly not representative	Formaldehyde (one 2-hr sample) in the bedroom at pillow height. Also measured guanine in bedroom (house dust mites), and	Spirometry and peak flow protocol described; FEV_1 (percent predicted accounting for age, sex, and height).	Analysis did not account for high prevalence of asthma symptoms in study group; VOC concentrations were correlated and effects could not be separated	FEV_1 was percent predicted accounting for age, sex, and height; Kendall's rank correlation test	N=88	 Exposure: Most exposed to concentration <LOQ Study population selected for high prevalence of asthma symptoms; Possible

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	sample because study design selected 50% subjects with asthma symptoms (may respond differently to formaldehyde exposure)	room temperature, air humidity, VOCs, respirable dust, and CO_2 in living room and bedroom. Limited sampling period in closed residence with no point formaldehyde emissions; sampling and analytic protocols referenced (Andersson et al., 1981) LOQ 0.1 mg/m^3); Formaldehyde and Range <0.005−0.110 $\mu g/m^3$ (most <LOQ)	PEF measured twice per day for 7 d; constructed variable for PEF variability (assessed in asthma section)	from those of formaldehyde (No data presented)			confounding: Co-exposures
Wallner et al. (2012)	9 schools selected of 19 who volunteered;	Measurements of 252 chemicals in 9 home classrooms	Spirometry protocol described; percent of reference	Reference values based on gender, age, height, and weight of children;	Associations with lung function analyzed for 34 chemicals; no adjustment for	N=433	

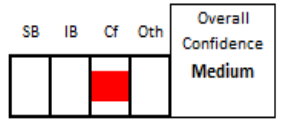
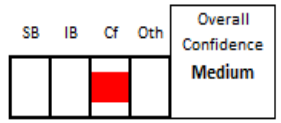
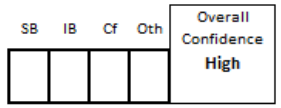
Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	72.7% participation	(exposed 6–7 hrs/d); 24 hr samples, 2 samples per classroom, 2 seasons; all students in class assigned the median chemical concentration; median 29.8 µg/m^3 (range 6.5–136.5 µg/m^3		regression analysis controlled for SES (education and occupation of parents, urban/rural, # smokers at home. No adjustment for other chemicals in classroom. Do not expect correlation between formaldehyde and PBDE congeners or phthalates in dust	multiple comparisons; multiple regression model, % change per 1 SD increase in formaldehyde (value of SD not reported).		 No adjustment for co-exposures in classroom that were also associated with pulmonary function, but correlation not anticipated
Occupational Studies							
Alexandersson et al. (1982)	All exposed workers employed >1 yr, recruitment from workers present on study day (healthy worker effect). Referents selected from plant	TWA personal sampling; 1 working day. Range in exposed 0.05–1.62 mg/m^3; referent not reported; although no measurements in referent, high	Spirometric measures (ATS methods); measured on Monday morning and after work in exposed; referents tested either in the morning or afternoon	Preshift variables compared to reference equations	Preshift values compared to predicted based on age, height, and gender evaluated within exposed and referent groups. SD not reported; difference across shift, compared mean values before and after	N=47 exposed; N=20 referent	Preshift  Concern for selection for healthy. *P*-values were reported Cross-shift 

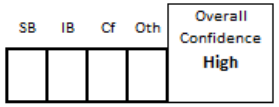
Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	employees not exposed to irritants; participation rate not reported. Cross-shift change not evaluated in referent	concentration in exposed allows assumption of an adequate exposure contrast for comparison of exposed and referent			shift in exposed (paired *t*-test) No comparison group		No comparison group
Alexandersson and Hedenstierna (1989); Alexandersson et al. (1982)	Possible selection for healthy during 4-yr follow-up; 13 exposed and 2 referents lost-to-follow-up; 13 exposed transferred to unexposed jobs	TWA using personal sampling among all exposed; 3–4 measurements of 15 min periods during 2 working d. Range in 1980 exposed 0.05–1.62 mg/m^3; referent not reported; Range in 1985 not reported. Sampled for dust. Although no measurements in referent, high	Spirometric measures (ATS methods); measured on Monday morning across shift in exposed; referents tested either in the morning or afternoon	Values compared to predicted normal based on age, gender, and height; analyses stratified by smoking status. Dust levels considered to be low.	Mean absolute value (SD) before work compared to predicted normal based on age, gender, and height in 1980 and 1984, and mean difference from predicted (SD) in 1984 by smoking status; 5-yr change corrected for age-dependent change; stratified by smoking. Mean change across shift (SD) stratified by smoking, no comparison group (low)	N=21 exposed; N=18 referent	Preshift  Concern for selection for healthy; small sample Cross-shift  No comparison group

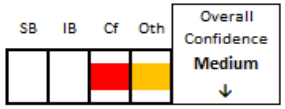
Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		concentration in exposed allows assumption of an adequate exposure contrast for comparison of exposed and referent.					
Alexandersson and Hedenstierna (1988)	Selection for healthy; evaluated employees present at work on study day	TWA using personal sampling, 3–4 15-min samples/ person; 1 working day. Range in exposed 0.12–1.32 mg/m^3; referent not reported; although no measurements in referent, high concentration in exposed allows assumption of an adequate exposure	Spirometry on Monday after two days unexposed and again at end of shift on second day. Half of referent tested before, and half tested after shift	Referents were "nonexposed" employees at same factory. All male, exposed slightly younger, 50% smokers; referent: 33% smokers. Analyses stratified by smoking status. Sampled for dust and solvents: Authors considered all exposures to be very low and not confounders	Mean values and difference from reference values by exposure group, and by smoking status among exposed. Change over 2 d by smoking status. Mean comparisons within exposure groups, Student's *t*-test	N=38 exposed; N=18 referent	Preshift  Concern for selection for healthy, small samples Cross-shift  No comparison group

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		contrast for comparison of exposed and referent.					
Gamble et al. (1976)	Of 68 workers exposed to hexa-methylene-tetramine-resorcinol resin, 52 (77%) completed questionnaire and lung function testing	Area samples	Spirometry protocol described	Referent matched by age, race, sex, shift, and job; Exposure to multiple chemicals	Exposure group defined by use of hexamethylene-tetramine-resorcinol resin, not formaldehyde	N=19 exposed; N=19 referent	 No quantitative analyses specifically for formaldehyde
Herbert et al. (1994)	Participation 98% in exposed, 82% in referent. Excluded accidental hydrogen sulfide exposure (n=14). Cross-shift change not evaluated in referent	TWA continuous sample in breathing zone; 5 sites, 2 d. Range in exposed 0.09–0.33 mg/m^3; **referent not reported**; sampled for dust. Although no measurements in referent, formaldehyde exposure not	Spirometric measures; best of 5 maneuvers, Snowbird criteria (Ferris, 1978); at start of work shift and after 6 hrs	Preshift comparisons adjusted for age, height, and smoking; not dust levels, which authors considered to be low	Exposed compared to referent using ANCOVA adjusting for age, height, and cigarette pack-years. Presented absolute values and *p*-values from ANCOVA. Unconditional logistic regression of FEV_1/FVC <75% controlling for age and cigarette pack-years. Presented odds ratios, 95% CI by smoking category.	N=99 exposed; N=165 referent	Preshift  Selection for healthy in prevalence study; possible irritant exposure in referent; co-exposure to dust Cross-shift  No comparison group

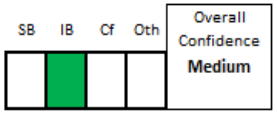
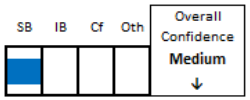
Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		expected for oil/ gas field workers; adequate exposure contrast likely for comparison of exposed and referent.			Presented absolute values of preshift and postshift with *t*-statistics and p-values; no comparison group		
Holmström and Wilhelmsson (1988)	100% participation; Possible differential imprecision of cumulative formaldehyde dose: formaldehyde levels estimated prior to 1979 when exposures were likely higher. Healthy workers	Area samples in one group, 1979−1984, personal samples (1−2 hrs) in 1985 in all groups. Estimated mean formaldehyde and dust exposure of every participant for each year of employment, dose-yrs. Range in Group #1 0.05−0.5 mg/m^3, Group #2 0.2–0.3 mg/m^3; referent mean 0.09 mg/m^3;	Spirometric measures (FVC, FEV_1/FVC) percent of expected normal based on age, sex, smoking, height, and weight.	Values compared to expected normal based on age, sex, smoking, height, and weight; respirable particulates measured but not adjusted for in analysis. Comparison groups: Formaldehyde only, formaldehyde and wood dust, referent group. Referent group was composed of administrative workers who may not be comparable to exposed.	Presented observed and expected values by exposure group, SD not reported. Statistical comparisons of observed and expected within exposure group (paired *t*-test); analyzed correlation with duration of exposure and cumulative dose but did not provide quantitative results	N=70 Group 1; N=100 Group 2; N=36 referent	 **Medium** Healthy workers; comparison groups selected from different source populations

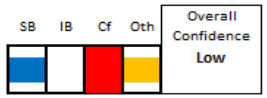
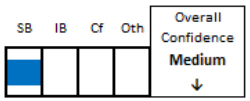
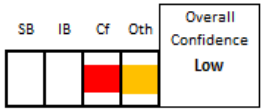
Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		adequate exposure contrast likely for comparison of exposed and referent.		Comparable smoking status between groups (data NR)			
Holness and Nethercott (1989)	Participants recruited from list of funeral homes, 86.6% participation; 79.8% of embalmers were active embalmers (healthy workers); community referent less similar?	2 area samples (impingers), during embalming, 30 to 180 min. Range in exposed 0.10−1.0 mg/m^3, referent mean 0.025 mg/m^3; adequate exposure contrast likely for comparison of exposed and referent.	Lung function as percent predicted; measured at initial assessment and before and after embalming procedure among exposed and before, and after a 2−3 hr period in referents.	Analyses adjusted for age, height, and pack-years smoked, referent may not be comparable for other possible confounders	Mean percent predicted (SD) presented by exposure group or by active or inactive embalmers, *p*-value from regression model adjusted for age, height, and pack-years smoked; percent change during embalming	N=84 exposed; N=38 referent	 Comparison groups selected from different source populations Change during embalming  comparison groups selected from different source populations
Horvath et al. (1988)	71% participation in exposed; 88% participation in referent. Age and sex distribution in participants	8-hr TWA using personal and area sampling on day of exam. Range in exposed 0.32 to 4.48 mg/m^3; referent	Spirometric measures (ATS methods); % predicted	Adjusted for age, sex, height, and smoking in analyses; particulates measured but not adjusted for in analysis. Smoking	Variables evaluated as percent of predicted normal; mean % predicted (SD) compared between exposure groups, *t*-test;	N=109 exposed; N=254 referent	Preshift  Cross-shift

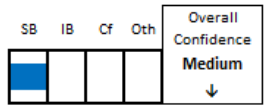
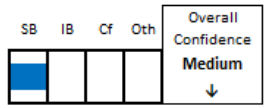
Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	similar to entire workforce in their respective companies. Evaluated and ruled out survivor bias using reasons for leaving employment among 54 former employees; evaluated characteristics of 30/45 nonparticipants who were younger and higher % male, with similar % smokers and mobile home residency.	0.037–0.15 mg/m^3; adequate exposure contrast likely for comparison of exposed and referent.		prevalence 53% in both groups; mean total particulates somewhat higher in referent. Other co-exposures not detected or a fraction of PEL (respirable particulates, phenol, CO, sodium hydroxide, NO_2 and acrolein).	multiple regression on log concentration adjusted for age, sex, height, and smoking; for cross-shift change, paired *t*-test (before and after) of percent predicted values		
Imbus and Tochilin (1988)	76% and 84.5% of employees tested at each plant	Area samples of formaldehyde and wood dust on same day as pulmonary testing. Sampling protocol (#	Spirometry protocol described (ATS); cross-shift change	Within person change; values presented as percent predicted; descriptive data on study group were not given.	Provided data, no statistical analyses presented	Plant A N=94; Plant B N=82	 Reporting deficiencies

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		samples and sampling period) not described. Range in exposed <0.012−0.074 mg/m^3		No unexposed referent group.			
Khamgaonkar and Fulare (1991)	Lab workers in college anatomy and histopathology departments; selected every 2nd person from occupational list.	Multiple 30-min area samples in the breathing zone in exposed (*N* = 43) and unexposed (*N* = 18) areas. Range in exposed 0.044−2.79 mg/m^3; referent mean 0.125 mg/m^3, range ND−0.64 mg/m^3; adequate exposure contrast likely for comparison of exposed and referent.	Spirometry protocol not described; measured on Monday. Selected every second person on list from each exposure group.	Comparison group matched by age and sex (*N* = 74). Comparable for mean height and weight; smoking prevalence: 54% exposed, 59% referent. Other exposures in lab	Mean absolute values (SD not reported) compared between exposed and referent; *p*-values reported	N=37 exposed; N=37 matched referent	 Possible exposures in referent that affect pulmonary function; exposure to formaldehyde in referent labs

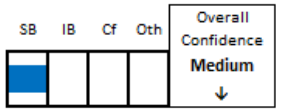
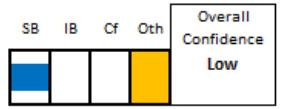
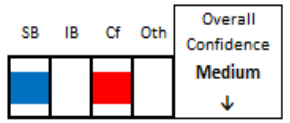
Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Kilburn et al. (1985b)	Concern for selection bias toward overestimating association. 41% participation, volunteers, nonrandom selection of participants in exposed. **Critical deficiency**	No formaldehyde concentration measurements. **Critical deficiency**	Spirometry protocol described; testing before and after work shift	Potential noncomparability of batt makers and administrative employees, calculated % predicted using reference population. Possible exposure to other contaminants among batt makers	Preshift absolute values and percent predicted, and postshift absolute values by smoking status (SD not reported) among batt makers and referent group	N=44 exposed; N=26 referent	 Low participation and nonrandom selection of exposed; no formaldehyde measurements and possible co-exposures
Kilburn et al. (1989a)	Attendees at 4 national conventions in 4 different cities between 1982 and 1986, compared to lung function in a Michigan population. Participation <40%; not clearly presented	Formaldehyde sampling in 10 labs in Los Angeles (not representative of entire sample); very wide range of concentration	Spirometry protocol described (ATS); percent of "referent" value	Questionable comparability to Michigan referent population; exposure both to formaldehyde and solvents; probable confounding by local air pollution in Anaheim, CA	Exposure group defined by histology technician; not specific to formaldehyde	N=280	 No quantitative analyses specifically for formaldehyde
Levine et al. (1984b)	94% participation among	No sampling measurements; Rank order	Spirometric measures	% predicted based on age and	Regression model of lung function in relation to	N=90	

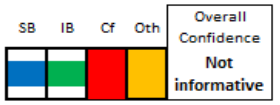
Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	morticians attending a required postgraduate course	using reported # embalmings. Comparison to funeral home records for 5 persons indicated # embalmings was over-reported.	(ATS methods), % predicted	height; all males and Caucasian	exposure rank, adjusted for age, height, pack-years. Table 6 in the paper: mean % predicted (SD) comparing low and high rank category by smoking status, low and high rank matched by age, Student's *t*-test		 Uncertainty regarding assignment to exposure rank
Löfstedt et al. (2009)	86% participation in exposed and 69% participation in referent. **Healthy survivor effect**	Personal samples on all exposed participants over a single 8-hour shift on same day as lung function testing. Range in exposed 0.014–1.6 mg/m^3; **referent not reported;** major exposure was to isocyanates, low correlation with formaldehyde concentrations	Spirometry protocol described (ATS methods), cross-shift change, percent predicted using Swedish reference; testing on day after 2 unexposed days	Referent from the same industry; older age and smoking prevalence higher in exposed. Important confounders addressed in analysis.	Regression models of association of change over shift with log formaldehyde level among exposed, adjusted for smoking on test day and co-exposure to ICA or MIC (in two models); compared mean change in % predicted across shift between exposed and referent	N=64 exposed; N=134 referent	Cross-shift  Healthy survivor effect.

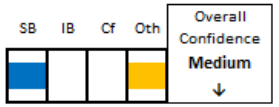
Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Löfstedt et al. (2011) (follow-up of Lofstedt (2009))	90% participation in exposed and referent. Evidence of survivor bias: prevalence of childhood allergy lower among exposed in 2005 (4% versus 31%). Higher prevalence of nasal symptoms among referents in 2005.	Personal samples on all exposed participants over a single 8-ur shift on same day as lung function testing. Range in exposed in 2001: 0.014−0.44 mg/m^3, range in exposed in 2005: 0.01−0.19 mg/m^3; referent not reported	Spirometry protocol described (ATS methods), cross-shift change, percent predicted using Swedish reference; testing on day after 2 unexposed d	Referent from the same industry; comparable for age; smoking prevalence and work duration higher in referent. Exposure to formaldehyde, MIC and ICA among exposed; correlation between formaldehyde and isocyanates low. **Analysis within each exposure group**	Compared preshift percent predicted values (SD) from 2001 and 2005 and change between the years (SD) within exposed and referent (Student's *t*-test). Multiple regression of changes in percent predicted across shift adjusted for MIC, formaldehyde, smoking (pack-years), and childhood allergy; authors stated no significant association but quantitative results were not reported.	N=25 exposed; N=55 referent	Preshift 2001 to 2005  Limited sample size to detect small changes between 2001 and 2005; concern for survivor bias; Co-exposure to MIC & ICA in exposed—unable to differentiate for comparisons of change from 2001 to 2005. Cross-shift 
Main and Hogan (1983)	All administrative personnel (exposed) and all workers on payroll (police personnel) who	Three 1-hour area samples (impingers), 4 occasions (August, September, December,	Spirometric measures (ATS methods); Percent predicted	Percent predicted, stratified by smoking status; potential dissimilarity between	Percent predicted by exposure group and smoking status; t statistic and *p*-value presented	N=14 exposed; N=17 referent	Preshift  Comparison groups selected from different sources (possible

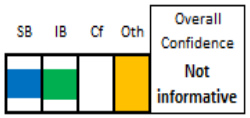
Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	did not work in trailers (referent) who were still employed at end of 34–mo period. **Comparison groups not similar**	April) always on a Monday. Range in exposed 0.15–1.97 mg/m^3; limited sampling period in closed structure with no point formaldehyde emissions; sampling and analytic protocols referenced; **referent not reported**		administrative employees and police officers; ETS more common among referent			unmeasured confounding), ETS in referent; small sample size (low sensitivity)
Malaka and Kodama (1990)	Participation 93%; current workers. Healthy survivor effect	Personal and area sampling, duration not reported; JEM (cumulative measure); range in exposed 0.27–4.28 mg/m^3, referent 0.004–0.09 mg/m^3; sampled for dust; adequate	Spirometric measures (ATS methods); % predicted and absolute values tested on Monday and cross-shift	Referent from same company; matched on age, ethnicity and smoking; analyses adjusted for age, height, weight, cigarettes per day, and dust.	Percent predicted by category of cumulative exposure (none, low, high) using ANCOVA; Linear regression of absolute value on cumulative exposure adjusted for age, height, weight, cigarettes/ day, and dust. Cross-shift change: means of absolute	N=93 exposed; N=93 referent	Preshift  Cross-shift 

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		exposure contrast likely for comparison of exposed and referent.			values compared before and afer shift in exposed and referent, paired *t*-test		
(Milton, 1996, 1314209@@author-year}	Evidence of selection of healthy workers (some refusals to avoid working in basement area); direction toward underestimation of effect	Personal sampling on each participant during 5−6 d of PEF measurement, 4 hrs on 2 d, same day as lung function testing; calculated 8-hr TWA. Range in exposed 0.0012−0.265 mg/m^3	Spirometry protocol described (ATS criteria); tested before and after work after 2 d off work and 2 other work d. PEF using mini-Wright peak flow meter, measurements 5 per day during and off work, 6 d at work and 4 d off. Self-reported PEF correlated with spirometric PEF (88 person-days before (r = 0.91) and after (r = 0.93) shift	Within person change, cross-over design, also adjusted for night shift and PEF at home, multiple exposures including to endotoxin, phenol resin, and formaldehyde. Concentrations were correlated—difficult to differentiate individual risk	PEF variability (high minus low for the day as percent of mean over all days). Linear regression of FEV_1 and FVC and home amplitude percent mean PEF adjusted for smoking, pack-years of cigarettes, and years since start of exposure. Cross-shift PEF and overnight PEF, logistic regression of ≥5% decline in PEF or linear regression of change in PEF on natural log of formaldehyde; models were GEE to account for repeated measures	N=37	 Correlated co-exposures

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Neghab et al. (2011)	Participation 100%. Cross-shift change not evaluated in referent. **Healthy survivor effect**	Area samples (40 min, *N* = 7) in 7 workshops and 1 area sample in office area. Range not reported, mean (SD) 0.96 (0.49); **referent not reported;** adequate exposure contrast likely for comparison of exposed and referent.	Spirometric measures (ATS methods); testing before and at end of shift on first working day of the week; percent predicted	Referent from the same industry and comparable socioeconomic and demographic status; % predicted based on age and height; all male	Preshift values (percent predicted) (SD) compared between exposed and referent (Student's *t*-test), Pre- and postshift percent predicted compared (paired *t*-test); Regression models of lung function and association with duration of exposure adjusted for age, height, weight, and smoking	N=70 exposed; N=24 referent	Preshift  Healthy worker survival. Obtained additional information from author to clarify results. Cross-shift  No comparison group
Nunn et al. (1990)	Follow-up complete (1980–1985) for 76% of exposed and 74% of referent. Attempted to include former employees; evidence of survivor bias	Area samples (1–6 hrs) 1979–1985, personal samples for representative set of exposed workers, 1985–1987, estimated prior to 1979. Range in exposed	FEV_1 values (FEV_1/height^3), adjusted for height	Referent group from same factory but exposed to other potential irritants (phenolic and epoxy resins, carbon fibers) and phenol- and urea-formaldehyde.	Regression of FEV_1/height^3 on time of screening visit for each worker, adjusting for age in 1980, smoking status in 1980 and 1985, maximum and mean exposure rank, and total duration of	N=125 exposed; N=95 referent	 Concern for selection bias: loss to follow-up higher among exposed with low lung function compared to referent; referent exposed to other potential irritants.

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		0.1–2.46 mg/m^3 and above. Uncertainty regarding formaldehyde levels in referent not reported		Stratified results by smoking	exposure. Presented mean slope (95% CI) by exposure (exposed and referent), and smoking status		
Ostojić et al. (2006)	16 physicians and lab technicians exposed daily in pathology/ anatomy lab (employed >4 yrs), source of referent not described (all male, matched for age and height)	Assessment of formaldehyde exposure was not described. No concentration data reported; exposed defined by work in pathology/ anatomy lab	Spirometry protocol described; morning measurements; percent expected	Referent matched by age and stature, all nonsmokers	Compared percent predicted (mean, SD) in exposed and referent using Student's *t*-test	N=16 exposed; N=16 referent	 Reporting deficiencies.
Pourmahabadian et al. (2006)	Selection and participation of study groups not described.	Area samples, 8-hr average, not measured in referent	Spirometry protocol not described	Differences by group for age, length of service, height, sex, education, and smoking; no adjustment for age, height, sex, weight, or smoking	Absolute values preshift and postshift (mean, SD), and mean difference across shift (SD) compared between exposed and referent using t-test. No adjustment for	N=124 exposed; N=56 referent	 Reporting deficiencies; concern for confounding.

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
					age, height, sex, weight, or smoking		
Schoenberg and Mitchell (1975)	Participation 94%; current workers. Healthy survival effect	Formaldehyde measurements taken by insurance company during same month; 0.5–1 mg/m^3; 3 breathing zone samples, 10.6–16.3 mg/m^3; exposed categorized by duration; additional exposure to phenol (5–10 mg/m^3; OSHA PEL 19 mg/m^3). Concentrations for "never on line" not reported; adequate exposure contrast likely for comparison of exposed and referent.	Spirometric measures; measured before and after shift on Monday and Friday.	% predicted based on age, height, and gender; standardized for 15 pack-years cigarette smoking; multiple exposures (phenol)	Compared % predicted (adjusted for cigarette smoking) across categories of duration	N=48 exposed; N=15 referent	 Healthy survival effect. Multiple exposures: formaldehyde, phenol. Phenol is an irritant but may not be associated with pulmonary function at these levels. Small sample size.

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Sripaiboonkij et al. (2009)	100% and 71% participation in exposed and referent	Area samples; #, dates and protocol not described	Spirometry protocol described	Models adjusted for age, sex, education, smoking, and ETS. Co-exposures to other irritants (glass microfibers) and sensitizers (phenol resin, mineral oils)	Exposure group defined by glass microfibers or sensitizing agents; not specific to formaldehyde	N=19 exposed; N=159 referent	Not Informative 
Tanveer et al. (1995)	49 male workers exposed to formaldehyde resins (mean duration 15.6 yr) and 29 male referents (security and administrative staff). Recruitment and participation not described. Healthy survivor effect possible	8-hr TWA 0.03 mg/m^3; exposure protocols and measurements not described. (*concerned that TWA value may be a typo because of comment in discussion stated that findings by Dally et al. at 0.33–1.7 ppm supported by this study at 0.03 mg/m^3*)	Respiratory questionnaire, standardized MRC, and spirometry (ATS protocol); baseline in morning and at end of workshift (cross-shift measured in 31 exposed and 22 referent)	Exposed and referent comparable for age, height, smoking, and alcohol; co-exposures not discussed	Compared preshift % predicted, exposed and referent, means, by smoking status and duration of exposure, Student's *t*-test; compared cross-shift change	N=49 exposed; N=29 referent	 Unable to assess exposure assessment or recruitment and selection protocol; Concern for selection for healthy

Supporting Material for Hazard Analyses of Pulmonary Function

Table A-45. Formaldehyde effects on pulmonary function in controlled human exposure studies

Study and design	Results
Medium Confidence (Randomized, results fully reported)	
References: Schachter et al. (1986); Witek et al. (1986) **Population**: N = 15 healthy, age 18–35 yrs, N=15 asthmatic, age 22 ± 5 yrs, all nonsmokers. **Exposure:** 40 min; Clean air and 2 ppm (2.46 mg/m^3)[a] **Protocol:** Random assignment to order of exposure, double blinded. Two dose levels, four exposure conditions, 2 d at rest and 2 d with exercise segment (10 min, at 10 min into the exposure period), separated by 4 d. Testing at baseline, and at 4 times during 40-min exposure, and 10 and 30 min postexposure. Change from baseline tested using "standard test" and Bonferroni adjustment.	No decrements in percent change from baseline in resting protocol; FVC, FEV_1, MEF50% (shown below), MEF40% or R_{aw}. Exercise protocol showed decrement in MEF50% 30 min after exposure end. **Percent Change from Baseline (Mean±SD)** Clean Air / 2 ppm **FVC (L)** **During exposure (@ 40 min.)** rest −1.14 ± 4.8 / −0.99 ± 3.5 exercise 1.6 ± 7.7 / 0.17 ± 6.2 **FEV_1 (L)** rest −0.41 ± 5.0 / 1.65 ± 4.5 exercise 4.87 ± 8.3* / 4.56 ± 5.3** **MEF50% (L/sec)** rest 2.74 ± 4.4 / 7.4 ± 5.0* exercise 8.72 ± 12.6 / 8.8 ± 8.1** **FVC (L)** **30 min. postexposure** rest 0.31 ± 5.1 / 1.75 ± 3.5 exercise −2.53 ± 5.4 / −0.25 ± 5.6 **FEV_1 (L)** rest 0.5 ± 4.7 / −1.15 ± 5.3 exercise −0.37 ± 4.5 / 1.76 ± 4.91 **MEF50% (L/sec)** rest −0.87 ± 5.4 / 2.65 ± 8.1 exercise 1.07 ± 5.3 / −5.74 ± 5.4** *p <.05; **p <.01
Reference: Schachter et al. (1987) **Population**: N = 15 healthy hospital laboratory workers routinely exposed to HCHO as part of their job, age 32 ± 11.3 yrs, 33.3 % male, N = 2 smokers. **Exposure:** 40 min; clean air and 2.0 ppm (2.46 mg/m^3)[a] **Protocol:** Random assignment to order of exposure, double blinded. Two dose levels, four exposure conditions, 2 d at rest and 2 d with exercise. One 10-min exercise segments at 5 min into the 40-min exposure period. Testing at baseline, and at 4 times during exposure, and 10 and 30 min postexposure. Percent change from baseline tested using one sample *t*-test with Bonferroni adjustment.	**Percent Change from Baseline (Mean±SD)** Clean Air / 2 ppm **FVC (L)** **During exposure (@ 40 min.)** rest −1.64 ± 5.67 / −1.30 ± 3.64 exercise −1.32 ± 6.94 / −1.60 ± 6.03 **FEV_1 (L)** rest −1.25 ± 5.25 / -2.05 ± 3.62 exercise −0.67 ± 6.33 / −1.56 ± 6.02 **FVC (L)** **30 min. postexposure** rest 0.68 ± 4.13 / −0.54 ± 2.51 exercise 0.30 ± 4.58 / −0.07 ± 4.25 **FEV_1 (L)** rest 1.94 ± 5.85 / −0.95 ± 3.0 exercise 0.62 ± 3.81 / 0.23 ± 4.2

Study and design	Results
Reference: Green et al. (1987) **Population**: n = 22, mean age 26.9 ± 3.6 yr, nonsmoking, no history of allergies or hay fever; gender not reported. **Exposure:** 60 min, clean air or 3.01 ± 0.01 ppm [3.7 ± 0.01 mg/m^3][a] **Protocol:** Random assignment to order of exposure; single blinded. Two 15-min exercise segments at 15 and 45 min into the 60-min exposure period. Testing before and during exposure period (approximate 15 min intervals); paired *t*-test comparing ratio of exposed value at time(n) to time(0) to ratio of clean air value at time(n) to time(0).	Declines evident at 47 min, Statistically significant decrements measured in several endpoints at 55 min. **Absolute values at 55 min exposure** Clean air / 3 ppm FVC: 5.04 ± 0.15 / 4.92 ± 0.15* FEV_1: 4.29 ± 0.12 / 4.15 ± 0.13* FEV_3: 4.93 ± 0.15 / 4.80 ± 0.15* FEF_{25-75}: 4.74 ± 0.25 / 4.56 ± 0.29 *p < 0.02, paired t-test
Reference: Green et al. (1989) **Population**: N = 24, 14 women and 10 men, age 18−35 yrs, nonsmoking, no history of asthma, no medications, FVC >80%, FEV/FVC >75%. **Exposure:** 2 hr, clean air, 3 ppm [3.69 mg/m^3][a], 0.5 mg/m^3 ACA (activated aerosol carbon), 3 ppm plus 0.5 mg/m^3 ACA. **Protocol:** Randomized block design with 4 2-hr exposure conditions, one per week; double blinded. Four 15-min exercise segments at 15, 45, 75, and 105 min into the 2-hr exposure period. Spirometric testing before and during exposure period (5 times). PEF at 2 hrs, and hourly intervals for 8-hrs postexposure, and at 12 and 16 hrs postexposure.	Results presented in graphs for FEV_1, FVC, FEF_{25-75}, and FEV_3. During exposure to formaldehyde + ACA, statistically significant changes were measured in FVC and FEV_3 at several intervals and decreased SG_{aw} was measured at the end of exposure; magnitudes of the changes were less than 10% of baseline. No statistically significant (p >0.05) effects were observed on FVC, FEV_1, or FEV_3, at any of 5 intervals during 2-hr exposures; for formaldehyde only exposure, statistically significant decrements were observed for FEF_{25-75} and SGaw at 50 and 80 min, magnitudes of the changes were 3−5%, compared with baseline.
Low Confidence (Incomplete reporting of results, or blinding not described with multiple exposure levels)	
References: Andersen and Molhave (1983); Andersen (1979) **Population**: N = 16 healthy students, age 30−33, 68.8 % male, 31.2% smokers **Exposure:** 5 hours; 0.3, 0.5, 1.0, and 2.0 mg/m^3 **Protocol:** Formaldehyde exposure order determined by Latin square design; blinding not described. Groups of 4 over 4 d; testing before (during 2 hrs clean air) and 2 times during exposure. No exercise component.	No change in FVC, FEV_1, or FEF_{25-75}; data presented in graphs Visual inspection indicates decrease in VC at 1 and 2 mg/m^3, FEF_{25-75} at 0.5 mg/m^3 (not statistically significant).
Reference: Kulle et al. (1987) **Population**: Group 1 (N = 10), Group 2 (N = 9), nonsmoking healthy, age 26.3 ± 4.7 yrs, 53% male. **Exposure:** 3 hr, Group 1: 0.0, 0.5, 1.0, or 2.0 ppm at rest (0.0, 0.62, 1.23, 2.46 mg/m^3)[a] at rest, and an additional 2.0 ppm with exercise; Group 2: 0.0, 1.0, or 3.0 ppm (0.0, 1.23, or 3.69 mg/m^3), and an additional 2.0 ppm with exercise. **Protocol:** Exposure order randomly assigned; blinding not reported. 3-hr exposures each week, at same time on 5 occasions. 8-min exercise segment every half hour during 2 ppm exposure. Pulmonary function tests (FVC, FEV_1, FEF_{25-75} and	No change in pulmonary function (means by testing time, no SD presented).

Study and design	Results
SGaw) at 0, 30, 60, 90, 120, 150, and 180 min during exposure, and 24 hrs postexposure.	
Reference: Lang et al. (2008) **Population**: N=21, age 19 – 39 years, nonsmoking, healthy volunteers. **Exposure:** 4 hours, clean air, 0.15, 0.3 and 0.5 ppm (0.0, 0.19, 0.37, and 0.62 mg/m^3)[a]; additional 0.3 and 0.5 ppm with peaks up to 1.0 ppm (1.23 mg/m^3)[a]; additional 0.0, 0.3, and 0.5 ppm with ethyl acetate to "mask" formaldehyde. **Protocol:** Exposure order randomly assigned; double blinded. Ten 4-hour exposure conditions, one per day, over 10 days. Airway resistance (R_{tot}, PEF, FEV_1, FEF_{25-75}, and SGaw measured on first exam and on first and last exposure day, pre and post exposure. No exercise component.	No statistically different differences between baseline Day 1 and postexposure on Day 10 (data not presented).
Low Confidence (No randomization; blinding not discussed)	
Reference: Day et al. (1984) **Population**: 2 groups of 9 adults each. Group 1, N = 9, adversely affected (nonrespiratory) by HCHO fumes emitted by urea foam insulation (UFFI) in their homes. Group 2, N = 9, not affected by UFFI present in their homes, or volunteer with no UFFI exposure. Descriptive data on study subjects was not presented. **Exposure:** 1.5 hrs in chamber, 1.0 ppm (1.23 mg/m^3)[a], 0.5 hr under hood, 1.2 ppm (1.48 mg/m^3)[a]; no clean air control. **Protocol:** Testing before, after, and 6.5 hrs after exposure. No exercise component.	No change in FVC, FEV_1, or FEF_{25-75} (mean ± SD) paired *t*-test
Reference: Sauder et al. (1986) **Population**: n = 9, mean age 26 ± 3.6 yrs, healthy, non allergic (for 6 wks prior to test), nonsmokers. **Exposure:** 3 hrs; 0, 3 ppm (3.69 mg/m^3)[a] **Protocol:** Nonrandom assignment; blinding not described. 8-min bicycle exercise followed by spirometry measurements after each 30-min interval during 3 hr exposures. First day clean air only, second day 3 ppm formaldehyde. Testing again after 24 hrs. Repeated measures ANOVA	**Clean air** / **3 ppm** 30 minutes FVC 4.61 / 4.62 FEV_1 3.98 / 3.90* FEF_{25-75} 4.46 / 4.16** 180 minutes FVC 4.71 / 4.68 FEV_1 4.02 / 3.99 FEF_{25-75} 4.45 / 4.48 *p <0.05, ** p <0.01, paired t-test Statistically significant decreases in FEV_1 (2%) and $FEF_{25\%-75\%}$ (7%) after first 30 minutes; Range in response: FEV_1 −5% to +1% FEF_{25-75} −14% to +2% No other changes during exposure or 24 hrs after.

[a]Concentrations reported by authors as ppm or ppb converted to mg/m^3.

Study summaries describing change in pulmonary function measures during a work shift or anatomy lab session

Appendix Figures A-24–A-26 present study findings for three spirometry measures, FEF_{25-75}, FEV_1, and FVC, and study details are summarized in Appendix A Table A-46. For each measure,

the mean difference across a work shift or lab session in exposed and referent groups (when reported) is plotted with error bars depicting the standard error. Separate graphs depict the mean before and after difference expressed as absolute value (e.g., FEV_1 in liters) or percent predicted. The third plot shows results for studies that reported changes as a percent of the baseline value.

Reference	Setting	Referent	Confidence
Malaka, 1990, N = 55	Wood products	N = 50	Medium
Alexandersson, 1989, N = 21	Wood products	Not measured	Low
Horvath, 1988, N = 109	Wood products	N = 254	High
Alexandersson, 1988, N = 38	Wood products	Not measured	Low
Alexandersson, 1982, N = 47	Wood products	Not measured	Low
Khaliq, 2009, N = 20	Anatomy lab	No referent	Low
Uba, 1989, N = 96	Anatomy lab	Week 2 vs baseline day	High

Reference	Setting	Referent	Confidence
Binawara, 2010, N = 80	Anatomy lab	No referent	Low

Reference	Setting	Referent	Confidence
Akbar-Khanzadeh, 1997, N = 50	Anatomy lab	N = 36	Low
Akbar-Khanzadeh, 1994, N = 34	Anatomy lab	N = 12	Medium

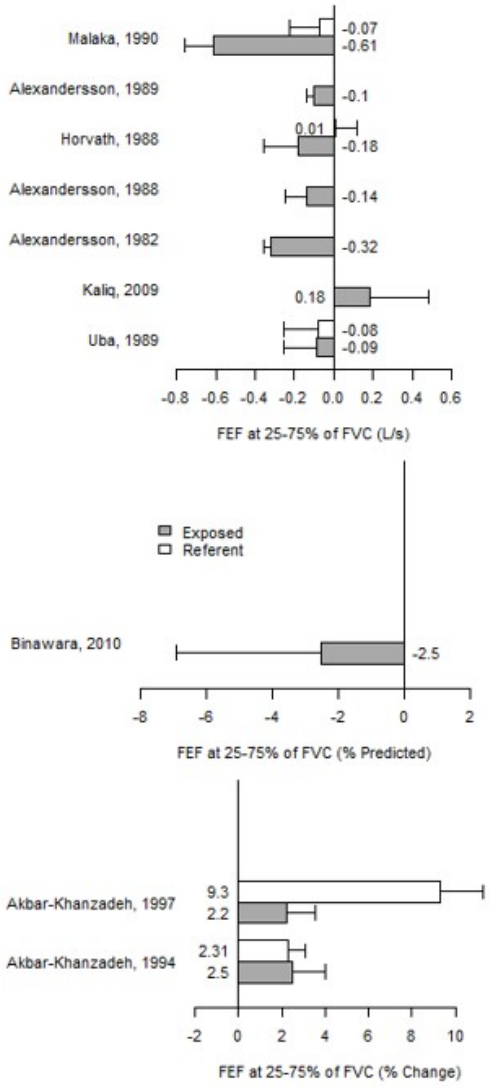


Figure A-24. Plots of change in FEF at 25–75% of FVC across a work shift or anatomy lab session by study with study details. The difference in reported means before and after shift or lab as either liters/second or % predicted are shown, and percent change in FEF across the lab was reported by two studies (3rd panel). Mean difference or percent change and SE are shown. These were calculated by EPA when not reported using SD for before and after means.

Reference	Setting	Referent	Confidence
Malaka, 1990, N = 55	Wood products	N = 50	Medium
Herbert, 1994, N = 99	Wood products	Not measured	Low
Alexandersson, 1989, N = 21	Wood products	Not measured	Low
Horvath, 1988, N = 109	Wood products	N = 254	High
Alexandersson, 1988, N = 38	Wood products	Not measured	Low
Alexandersson, 1982, N = 47	Wood products	Not measured	Low
Khaliq, 2009, N = 20,	Anatomy lab	No referent	Low
Chia, 1992, N = 13	Anatomy lab	Not measured	Low
Uba, 1989, N = 96	Anatomy lab	Week 2 vs baseline day	High

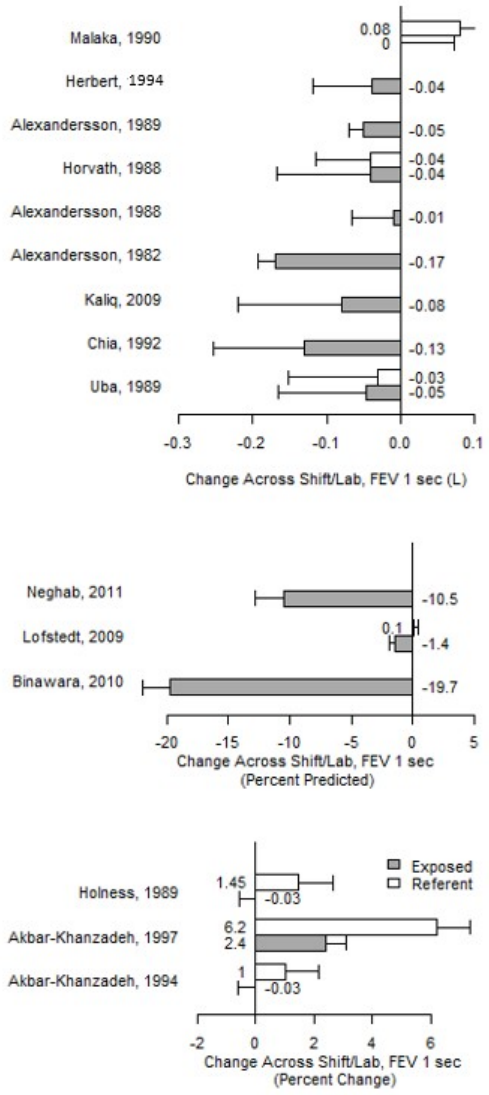


Figure A-25. Plots of change in FEV1 across a work shift or anatomy lab session by study with study details. The difference in reported means before and after shift or lab as either liters or % predicted are shown, or percent change in FEV1 across the lab. Mean difference or percent change and SE are shown. These were calculated by EPA when not reported using SD for before and after means.

Reference	Setting	Referent	Confidence
Malaka, 1990, N = 55	Wood products	N = 50	Medium
Herbert, 1994, N = 99	Wood products	Not measured	Low
Alexandersson, 1989, N = 21	Wood products	Not measured	Low
Horvath, 1988, N = 109	Wood products	N = 254	High
Alexandersson, 1988, N = 38	Wood products	Not measured	Low
Alexandersson, 1982, N = 47	Wood products	Not measured	Low
Khaliq, 2009, N = 20,	Anatomy lab	No referent	Low
Chia, 1992, N = 13	Anatomy lab	Not measured	Low
Uba, 1989, N = 96	Anatomy lab	Week 2 vs baseline day	High

Reference	Setting	Referent	Confidence
Neghab, 2011, N = 70	Chemicals	Not measured	Low
Lofstedt, 2009, N = 64,	Chemicals	N = 134	Medium
Binawara, 2010, N = 80	Anatomy lab	No referent	Low

Reference	Setting	Referent	Confidence
Holness, 1989, N = 22	Embalming	N = 13	Medium
Akbar-Khanzadeh, 1997, N = 50	Anatomy lab	N = 36	Low
Akbar-Khanzadeh, 1994, N = 34	Anatomy lab	N = 12	Medium
Demographic information for Holness, 1989 are for entire study groups.			

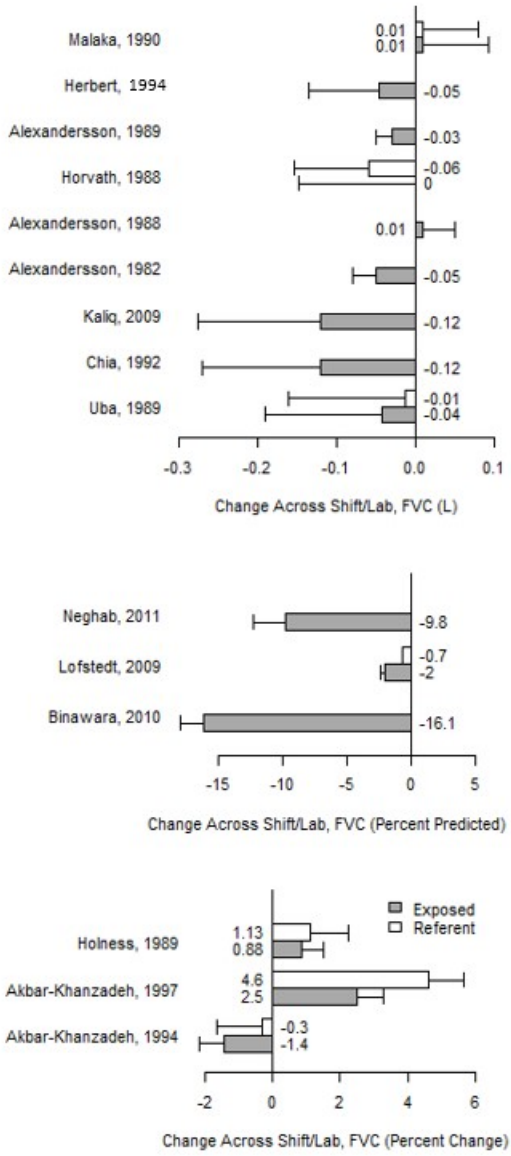


Figure A-26. Plots of change in FVC across a work shift or anatomy lab session by study with study details. The difference in reported means before and after shift or lab as either liters or % predicted are shown, or percent change in FVC across the lab. Mean difference or percent change and SE are shown. These were calculated by EPA when not reported using SD for before and after means.

Table A-46. Study details for references depicted in Figures A-24 – A-26

Study information	Group characteristics	Measures reported/ analysis
Occupational studies		
(Neghab et al., 2011) Resin production	Exposed: N = 70, male, age 38 yr, 24% smokers; Referent: Not measured	FEV_1, FVC, FEV_1/FVC, PEF Mean values (percent predicted) before and after shift compared (paired *t*-test) in exposed

Study information	Group characteristics	Measures reported/ analysis
Confidence: Low (No comparison group)		
(Löfstedt et al., 2009) Chemical company Confidence: Medium (Healthy survivor effect)	Exposed: N = 64, 89% male, age 44 yr, 25% smokers; Referent: N = 134, 88% male, age 40 yr, 22% smokers	VC, FEV_1 Compared mean difference across shift (percent predicted) between exposed and referent (regression); association with formaldehyde adjusting for isocyanate levels and smoking (regression)
(Malaka and Kodama, 1990) Plywood manufacture Confidence: Medium (healthy survivors)	Exposed: N = 55, male, age 27 yr, 53% smokers; Referent: matched by age, ethnicity and smoking; N = 50, male, age 29 yr, 53% smokers	FEV_1, FVC, FEV_1/FVC, FEF_{25-75} Mean values before and after shift compared (paired *t*-test) in exposed and referent
(Herbert et al., 1994) Particle board manufacture Confidence: Low (No comparison group)	Exposed: N = 99, sex NR, age 35 yr, 52% smokers; Referent: Not measured	FEV_1, FVC, FEV_1/FVC Mean values before and after shift compared (paired *t*-test) in exposed
(Alexandersson and Hedenstierna, 1989) Cabinet manufacture, 5-yr follow-up of (Alexandersson et al., 1982) Confidence: Low (No comparison group)	Exposed: N = 21, male, age 37 yr, 48% smokers; Referent: Not measured	FEV_1, FVC, FEV_1/FVC, FEF_{25-75} Mean values before and after shift compared, stratified by smoking status (paired *t*-test) in exposed
(Holness and Nethercott, 1989) Funeral workers (embalming) Confidence: Medium (comparison groups selected from different source populations)	Exposed: N = 22, 89% male, age 32 yr, 50% smokers; Referent (community volunteers): N = 13, 84% male, age 28 yr, 37% smokers (Demographic information for are for entire study groups)	FEV_1, FVC, FEF_{50}, FEF_{75} Compared mean percent change during embalming (or after 2−3 hr) (percent predicted) between exposed and referent (regression adjusting for age, height, and pack-yr smoked
(Horvath et al., 1988) Particle board manufacture Confidence: High	Exposed: N = 109, 57% male, age 37 yr, 53% smokers; Referent (food processing): N = 254, 44% male, age 34 yr, 53% smokers	FEV_1, FVC, FEV_1/FVC, FEF_{25-75}, PEF Mean values before and after shift (percent predicted) compared (paired *t*-test) in exposed and referent; correlation with formaldehyde concentration
(Alexandersson, 1988) Wood products Confidence: Low (No comparison group)	Exposed: N = 38, male, age 34 yr, 50% smokers; Referent: Not measured	FEV_1, FVC, FEV_1/FVC, FEF_{25-75} Mean values before shift on first day and after shift on second day compared, stratified by smoking status (paired *t*-test) in exposed
(Alexandersson et al., 1982) Cabinet manufacture Confidence: Low (No comparison group)	Exposed: N = 47, male, age 35 yr, 51% smokers; Referent: Not measured	FEV_1, FVC, FEV_1/FVC, FEF_{25-75} Mean values before and after shift compared, stratified by smoking status (paired *t*-test) in exposed
Anatomy lab (dissection)		
(Saowakon et al., 2015) Anatomy course Confidence: Low (No comparison group)	N = 36, gender NR, age 19.8 yr, nonsmokers; no referent	FVC, FEV_1, FEV_1/FVC, FEF_{25-75}, PEF Mean values compared before and after dissection session (paired *t*-test) in exposed

Study information	Group characteristics	Measures reported/ analysis
(Binawara et al., 2010) Anatomy course Confidence: Low (No comparison group)	N = 80, male, age 20 yr, nonsmokers; referent: No referent	FEV_1, FVC, FEV_1/FVC, FEF_{25-75}, PEF Mean values (percent predicted) before and after shift compared (paired *t*-test) in exposed
(Khaliq and Tripathi, 2009) Anatomy course Confidence: Low (No comparison group; small sample size)	Exposed: N = 20, male, age 18 yr, nonsmokers; no referent	FEV_1, FVC, FEV_1/FVC, FEF_{25-75}, PEF Mean values before and after lab compared (repeated measure ANOVA) in exposed
(Akbar-Khanzadeh and Mlynek, 1997) Anatomy course Confidence: Low (Analyses did not account for possible acclimatization to formaldehyde over time)	Exposed: N = 50, 50% male, age 24 yr, nonsmokers; referent (physiotherapy students): N = 36, 24% male, age 24 yr, nonsmokers	FEV_1, FVC, FEF_{25-75} Compared mean percent change (standardized for baseline) over lab in exposed and referent (paired t-test); compared difference between groups (unpaired *t*-test)
(Akbar-Khanzadeh et al., 1994) Anatomy course, Confidence: Medium (Comparison groups dissimilar; small sample size in referent)	Exposed: N = 34, 71% male, age 26 yr, nonsmokers; referent: N = 12, 67% male, age 31 yr, nonsmokers	FEV_1, FVC, FEV_1/FVC, FEF_{25-75} Compared mean percent change (standardized for baseline) over lab in exposed and referent (paired *t*-test); compared difference between groups (unpaired *t*-test)
(Chia et al., 1992) Anatomy course Confidence: Low (No comparison group; small sample size)	Exposed: N = 13 male, n = 9 female, age NR, smoking NR; referent: Not measured	FEV_1, FVC (means adjusted for age and height); Mean values before and after lab compared (chi-square statistic)
(Uba et al., 1989) Anatomy course Confidence: High	Exposed: N = 96, 74% male, age 24 yr, nonsmokers; comparison: Cross-lab change week 2 vs. baseline day	FEV_1, FVC, FEV_1/FVC, FEF_{25-75} Mean percent change over lab session at 2 weeks compared to baseline (repeated measures ANOVA, adjusted for sex)

A.5.4. Immune-Mediated Conditions, Including Allergies and Asthma

Literature Search

A systematic evaluation of the literature database on studies examining the potential for respiratory and immume-mediated conditions, including allergies and asthma, in relation to formaldehyde exposure was initially conducted in October 2012, with yearly updates to September 2016 (see Section A.5.1). A systematic evidence map identified literature published from 2017 to 2021 (see Appendix F). The search strings used in specific databases are shown in Table A-47. Additional search strategies included:

- Review of reference lists in the articles identified through the full screening process,
- Review of reference lists in the 2010 draft Toxicological Review for Formaldehyde (U.S. EPA, 2010), and

- Review of abstracts (initial title search for formaldehyde, then abstract review) from 2005–2014 presented at International Society of Environmental Epidemiology annual meetings.

The focus of this review is on hypersensitivity (allergy) and on asthma; these are well-developed areas of research with respect to immune-related effects of inhalation exposure to formaldehyde. Within these areas, several different types of endpoints or outcomes have been examined. EPA included the following outcomes in studies in humans in this review:

- Prevalence of current allergy symptoms (nasal, ocular, or dermatologic), incidence of allergies, or skin prick tests in general population or occupational studies with inhalation exposure measures;

- Incidence of asthma (based on parent- or self-report of physician-diagnosis), prevalence of current asthma (based on various validated questionnaires or based on medical records), asthma control among people with asthma (based on questionnaires developed to assess markers of asthma morbidity such as symptoms, medication use and healthcare utilization); and

- Pulmonary function (standard spirometry) and bronchial challenge-airway reactivity tests among people with asthma; [pulmonary function studies in general (nonasthmatic) populations were reviewed in the "Pulmonary Function" section].

EPA considered "ever had asthma" to be of limited use in this review, as the formaldehyde measures available do not reflect cumulative exposures that could be related to cumulative risk, and thus EPA did not include studies limited to "ever had asthma."

Case reports of occupational asthma were not systematically reviewed, but selected references are included for illustration. Formaldehyde-specific antibodies were not examined, as there has been little evidence of effects; selected references are included for illustration.

Based on the ultimate conclusion that the toxicity studies in animals were most appropriately reviewed as mechanistic information (see Section 1.2.3 of the Toxicological Review), the experimental studies identified as a result of this literature search are evaluated and described as mechanistic studies related to noncancer respiratory health effects section (see Appendix A.5.6). In regard to the experimental studies identified by this literature search, particular attention (and inclusion/exclusion criteria applied in the HERO database) emphasized the identification of studies examining the following endpoints:

- Airway inflammatory responses to sensitizing antigens, such as bronchoconstriction and airway hyperresponsiveness. (Studies describing the development of immunological or allergy animal models were not included, however.)

- Biomarkers relating to potential mechanisms in animal toxicology studies, such as eosinophil infiltration, immunoglobulins (e.g., total or anti-allergen-specific IgE or IgG), and cytokines pertinent to hypersensitivity responses, and neurogenic mechanisms of airway inflammation.

- Note: contact dermatitis is a well-established effect from dermal exposure and the effects of dermal exposure are not a focus of this review; thus studies of contact dermatitis from dermal exposures are excluded from this literature search (and the literature search in Appendix A.5.6).

Inclusion and exclusion criteria for selection of studies are summarized in Table A-48 and Table A-49, respectively, for human and animal studies.

After compilation into a single database and electronic removal of duplication citations, the 4,622 articles were initially screened within an EndNote library; the initial screening was based on title (3,409 excluded), followed by screening by title and abstract (1,046 excluded). Most of the exclusions at these stages were because the paper was not related to this review (e.g., studies of use of formaldehyde in vaccines, or studies of other chemicals) or were secondary data sources (reviews). Full text review was conducted on 167 identified articles. Most of the exclusions at this stage were because the study did not examine any of the selected outcome measures or did not conduct an analysis of formaldehyde. Four studies were excluded based on the aspects of the "comparison" criteria (e.g., limited exposure range):

- Smedje et al. (1997)—limited exposure range with 54% less than LOD (LOD 0.005, range <0.005 to 0.010 mg/m^3) [The follow-up study of this cohort, described in Smedje and Norback (2001) was not excluded because it included an additional measurement period and wider range of exposures.]

- Kim et al. (2007)—limited exposure range, with large percentage less than LOD (LOD 0.006, mean 0.007, maximum 0.016 mg/m^3)

- Zhao et al. (2008)—limited exposure range. The LOD was not reported but the minimum and maximum values were reported as 0.001 and 0.005 mg/m^3; this maximum is lower than the LOD in most studies. Technical difficulties led to the exclusion of measures from 14 of the 46 classrooms, but the authors did not comment on the unusual finding of higher levels in outdoor compared to indoor measures. [The corresponding author did not respond to an email inquiry asking for clarification regarding the exposure measures.]

- Chatzidiakou et al. (2014)—did not present an analysis of the effect of variability in formaldehyde within either urban or suburban setting, and the design did not allow for separation of effects of location from effects of formaldehyde.

The search and screening strategy, including exclusion categories applied and the number of articles excluded within each exclusion category based on the full text screening, is summarized in Figure A-27. Based on this process, 36 human studies and 16 animal-mechanistic studies were identified and evaluated for consideration in the Toxicological Review.

Table A-47. Summary of search terms – allergy-related conditions, including asthma

Database, Initial search date	Terms
PubMed 10/31/2012 No date restriction	formaldehyde and (asthma or wheeze or respiratory or allergy or immune or sensitization) NOT ("formalin test" OR "formaldehyde fixation" OR "formalin fixation" OR "formalin fixed" OR "formaldehyde fixed" OR "formalin-induced" OR "formalin-evoked")
Web of Science 11/5/2012 No date restriction	(TS=formaldehyde and TS=asthma) OR (TS=formaldehyde and TS=allergy) OR (TS=formaldehyde and TS=immune) OR (TS=formaldehyde and TS=respiratory) OR (TS=formaldehyde and TS=sensitization) OR (TS=formaldehyde and TS=wheeze)
Toxline 11/2/2012 No date restriction	formaldehyde @AND @OR (immune allergy asthma respiratory wheeze sensitization)

Table A-48. Inclusion and exclusion criteria for studies of allergy and asthma studies in humans

	Included	Excluded
Population	• Human	• Animals
Exposure	• Indoor exposure via inhalation to formaldehyde, measured in homes or schools or by personal monitors in general population studies • Occupational exposure settings (e.g., manufacture of pressed wood products)	• Not formaldehyde • Outdoor formaldehyde exposure • Dental-related exposures or cosmetic and other dermal-related exposures • Exposure via dialysis • Formaldehyde as fixative • Intervention studies in which formaldehyde and numerous other factors were simultaneously changed
Comparison	• Analysis of variation in risk in relation to variation in formaldedhye, specifcially: • at exposures above 0.010 mg/m^3 • across exposure range that spans at least 0.01 mg/m^3 (e.g., from 0.02 to 0.03 mg/m^3)	• Case reports (selected references used for illustration)
Outcome	• Allergy symptoms[a] • Skin prick tests • Incidence of specific allergies • Prevalence of current asthma[a] • Incidence of asthma • Asthma control or severity • Controlled exposure pulmonary function studies in people with asthma	• Sick building syndrome, sick building symptoms, chemical sensitivity studies • Contact dermatitis, eczema, or urticaria in studies of worker populations with likely dermal exposure • Formaldehyde-specific antibodies (FA-Ig) • Pulmonary function in controlled exposure studies in people without asthma [these studies are included in Section A.5.3. Pulmonary Function] • Lifetime prevalence of asthma (“Ever had asthma” or “ever had wheezing episode”)

	Included	Excluded
Other		• Reviews, reports, no abstract (title only), meeting abstract, methodology paper, formaldehyde used in vaccine preparation, other miscellaneous reasons—not on topic

[a]Based on the methods used in the American Thoracic Society questionnaire (Ferris, 1978) or subsequent instruments that built upon this work, such as the International Study of Arthritis and Allergies in Children (ISAAC) and European Community Respiratory Health Survey (ECHRS) questionnaires.

Table A-49. Inclusion and exclusion criteria for studies of hypersensitivity in animals

	Included	Excluded
Population	• Animals	• Humans
Exposure	• Inhalation route, formaldehyde	• Not formaldehyde • Oral or dermal exposure protocol
Comparison	• One or more exposure group compared to control	• No control group
Outcome	• Bronchoconstriction or airway hyperresponsiveness measures • Total or anti-allergen-specific IgE or IgG • Eosinophil infiltration in lung • Th2 cytokines (e.g., IL-4, IL-5)	• General chronic bioassay measures (e.g., organ weight, tumor incidence) • Host resistance assays • Antibody responses not involving respiratory sensitizers (e.g., sheep red blood cells, tetanus toxoid) • Dermal sensitization measures • In vitro studies, measures of inflammation and irritation (e.g., TNF-a, ROS), and formaldehyde-specific antibody studies were identified using a more specific search string in Section A.5.6.
Other		• Reviews, reports, meeting abstract, no abstract (title only), methodology paper

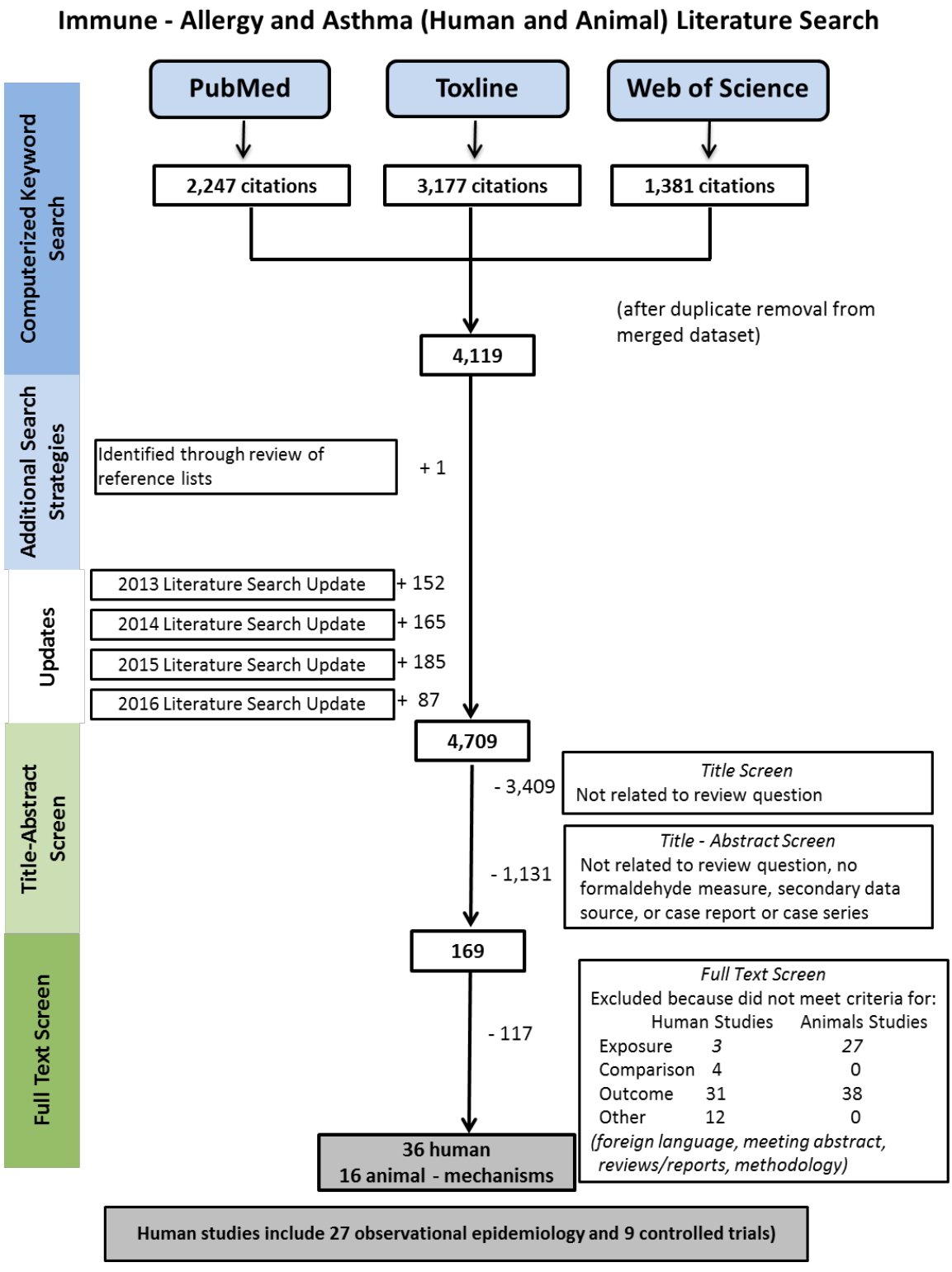


Figure A-27. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and respiratory and immune-mediated conditions.

Study Evaluations

The selected studies were evaluated using a systematic approach to identify strengths and limitations, and to rate the confidence in the results. Details of the evaluation considerations for the observational epidemiology studies of allergic response based on history of specific conditions or on skin prick tests, or asthma (current prevalentce, incidence, or asthma control) are described below, followed by a summary of the evaluation of controlled human acute exposure studies.

Observational Epidemiology Studies

Ascertainment of allergic sensitization and allergies

EPA consulted with a group of experts[15] regarding issues pertaining to ascertainment of allergy sensitization and allergies in epidemiology studies. The group was given extracted information regarding case ascertainment or outcome classification from 12 studies using questionnaire-based measures or skin prick tests; descriptive information about the study population (e.g., size, age, country) was also provided. The set included studies of formaldehyde and of other exposures, but the material did not include any information regarding results.

The experts raised several points about the types of measures and interpretations of these measures. The category includes allergic sensitization based on skin prick tests and history of allergy-related symptoms. Sensitization may be present without clinical symptoms, and symptoms may be present without a positive skin prick test. Thus, these address different (but overlapping) responses or conditions. The clinical expression of symptoms can be IgE-mediated or non-IgE mediated; in most cases studies are not designed to make this distinction. The experts recommended grouping the symptoms by site (i.e., nose and eyes; skin), and noted that food allergies constitute a different type of group.

Questionnaire-based ascertainments of nasal and ocular symptoms have been developed and widely used, for example in the International Study of Arthritis and Allergies in Children (ISAAC) (Asher et al., 1995). The additional ascertainment of seasonality and triggers can be helpful in distinguishing between allergic and nonallergic basis of the symptoms. When comparing specific types of self-reported allergies to specific types of positive skin prick tests, specificity of self-report is relatively high (approximately 90% or higher), but sensitivity is lower (ranging from 30–70%) (see for example see for example Lakwijk et al., 1998; Braun-Fahrländer et al., 1997; Dotterud et al., 1995). Limiting case ascertainment to physician-diagnosed allergies increases specificity but is considered to have low sensitivity because self-treatment with nonprescription medications is common. For studies of association, specificity is a more important consideration than sensitivity. It was also noted that validation of the questionnaire-based instruments is more established in Europe and the United States than in other populations.

Questionnaire-based ascertainments of atopic dermatitis or eczema have also been developed (Williams et al., 1996; Asher et al., 1995). These questionnaires focus on the extent, location, and itchiness of the rash and age at onset (typical onset before age 2 years). Specificity,

[15]Dr. Hasan Arshad, University of Southampton, Southamptom, United Kingdom; Dr. Peter Gergen, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland; Dr. Elizabeth Matsui, Johns Hopkins University, Baltimore, Maryland; Dr. Dan Norbäck, Uppsala University, Uppsala, Sweden; Dr. Matthew Perzanowski, Columbia University, New York City, NY.

compared to physician diagnosis, was high (>0.95) in school-age children (Williams et al., 1996) and in younger children (von Kobyletzki et al., 2013).

Based on the discussions with these experts, EPA made the following decisions:

- ISAAC questionnaires for rhinitis or rhinoconjunctivitis were considered to provide an adequate basis for case ascertainment in studies in Europe and the United States; in studies in other areas (i.e., areas that have not been included in ISAAC), specific mention of validation of the questionnaire was needed to receive a high confidence rating. Although the specificity of questions pertaining to rhinitis may be somewhat lower than the specificity of questions pertaining to rhinoconjunctivitis (Kim et al., 2012), this difference was not sufficient to conclude that the rhinitis questions should be viewed with lower confidence.

- EPA had lower confidence in the symptom ascertainment in Matsunaga et al. (2008) because this study was based on self-report of medical treatment (medication use) for atopic eczema and for allergic rhinitis in the past year, without clarifying the type of medication. EPA did not find studies examining the sensitivity or specificity of this question-based assessment with respect to ascertainment of allergy history.

- EPA had lower confidence in allergy ascertainment in Fransman et al. (2003) because the question included food as one of the types of allergies, and was not as specific regarding symptoms as the ISAAC-based questionnaires.

- Skin prick test protocols in the set of studies ranged from 5 to 12 allergens; EPA did not consider this difference to be sufficient to conclude that the protocols should be viewed with different levels of confidence.

Longitudinal studies can examine the initial manifestation of the response (sensitization or symptoms); cross-sectional studies can examine period-specific prevalence of allergies. Either question can be relevant when thinking about the influence of environmental exposures. For studies of incidence of allergies, the exposure measure should reflect a period before occurrence; for studies of the prevalence of allergy symptoms, the exposure measure should reflect the same period as the characterization of symptoms; for studies of allergy sensitization, the exposure measure should reflect the period before or during which sensitization occurs.

- In the only study of incident allergies (Smedje and Norback, 2001), the baseline assessment excluded children with a positive skin prick test. Measurements of formaldehyde in classrooms were taken at baseline and again two years later; the end of the follow-up period was two years after this measurement (4-year total follow-up). EPA considered this protocol to reflect a relevant exposure period.

- Because of questions regarding the relevant time window of exposure, EPA had lower confidence in skin prick test results for studies in adults than in children.

Ascertainment of asthma

EPA also consulted with a group of experts[16] regarding issues pertaining to ascertainment of asthma in epidemiology studies. This group was given extracted information regarding case ascertainment or outcome classification from 23 studies using questionnaire-based measures of asthma, some of which included a validation component. As with the other group, descriptive information about the study population (e.g., size, age, country) was also provided and the material did not include any information regarding results for formaldehyde or other exposures.

The experts raised several points about the ascertainment of asthma and the terminology used for different types of measures. Self- (or parent-) report of physician-diagnosed asthma can be reliably used in epidemiological studies of incidence of asthma, although this method can miss undiagnosed asthma. "Current" asthma, or prevalence of current asthma, is typically ascertained through a set of questions pertaining to symptoms or medication use over of period of time (e.g., last 12 months). A similar, but usually expanded, set of questions can be used to assess asthma control over a shorter period of time (e.g., 2–4 weeks). (Asthma control pertains to the extent to which symptoms can be reduced or eliminated with medication.) Asthma exacerbation is a term typically used in clinical trials and considers the need for using systemic corticosteroids. Most of the studies identified in the formaldehyde literature are studies of prevalence of current asthma.

Most of the studies identified in this review used a classification scheme based on the American Thoracic Society questionnaire (Ferris, 1978) or subsequent instruments that built upon this work, including the ISAAC and European Community Respiratory Health Survey (ECHRS) questionnaires. These questionnaire-based approaches have been found to have an adequate level of specificity and positive predictive value for use in etiologic research (Ravault and Kauffmann, 2001; Jenkins et al., 1996; Burney et al., 1989). The questionnaires typically use several questions to define current asthma based on symptoms relating to wheezing episodes or shortness of breath, reported history of asthma attacks, or use of asthma medication. Using the question "Has a doctor ever told you that you have asthma?" is a validated approach for the ascertainment of asthma incidence. As noted in the discussion of ascertainment of allergies, the questionnaires have been used in many studies but have not necessarily been validated in every population.

The age of study participants is an important consideration in the interpretation of various measures. Specificity of symptom questions is reduced in the very young (<5 years) because wheezing can occur with respiratory infections in infants and young children, and specificity is reduced at older ages (e.g, >75 years) because of the similarities in symptoms and medication use for chronic obstructive pulmonary disease and asthma (Abramson et al., 2014; Taffet et al., 2014).

Asthma can be atopic (allergic) or nonatopic. In the United States 1988–1994 NHANES data, 56% of self-reported physician diagnosed asthma cases had at least one positive skin prick test

[16]Dr. Lara Akinbami, U.S. Centers for Disease Control, Atlanta, Georgia; Dr. Peter Gergen, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland; Dr. Christine Joseph, University of Michigan, Ann Arbor, Michigan; Dr. Felicia Rabito, Tulane University, New Orleans, Louisiana; Dr. Carl-Gustaf Bornehag, Karlstad University, Karlstad, Sweden.

(Arbes et al., 2005). Thus, the delineation of asthma into these different groups can reduce some of the heterogeneity, but exclusion of either group may significantly reduce the sensitivity of case ascertainment.

Based on the discussions with these experts, EPA made the following decisions:

- ATS-based questionnaires or subsequent variations (ISAAC, ECHRS) for prevalence of current asthma that include questions on medication use and symptoms were considered to provide an adequate basis for case ascertainment in studies in Europe and the United States; in studies in other areas (i.e., areas that have not been included in ISAAC), specific mention of validation of the questionnaire was needed to receive this level of confidence.

- EPA had lower confidence in the asthma ascertainment in Matsunaga et al. (2008) because this study was based on self-report of medical treatment (medication use) for asthma in the past year. This ascertainment method may result in reduced sensitivity. The resulting prevalence of asthma based on this definition was lower than found in a study by Miyake (2011), which was conducted in a similar population (women enrolled in a pregnancy cohort in Japan) and used a broader definition based on symptoms and medication use [asthma prevalence 2.1% and 5.5%, respectively, in Matsunaga et al. (2008) and Miyake et al. (2011)]. With respect to specificity, this is a relatively young cohort (pregnant women, median age approximately 30 years), suggesting that chronic obstructive pulmonary disease would not be common.

- EPA had lower confidence in the asthma ascertainment in the study by Tavernier et al. (2006) because of low specificity of the classification. The experts noted that three of the five screening conditions were not specific to asthma (received more than three courses of antibiotics for upper or lower respiratory symptoms in the past 12 months, have history of fever or eczema, and family history of asthma in first degree relatives), and recommended excluding this study. However, because the study did meet EPA's initial inclusion criteria, EPA retained it but noted this limitation in the evaluation.

- Some studies included results for more than one asthma measure; in this assessment, EPA based its evaluation on outcomes that were defined over a recent time period (e.g., symptoms in the past 12 months) and did not include outcomes defined over a lifetime (e.g., ever had asthma). Studies that did not clearly delineate the time period of ascertainment were included, but EPA noted the lower confidence in these measures.

- Rumchev et al. (2002), a study of emergency room visits for asthma in children ages 6 months to 3 years was classified as not informative with respect to asthma. [NRC (2011) also recommended excluding Rumchev (2002) on the basis of the age distribution.] This study, in addition to two other studies that examined wheezing episodes among infants (Roda et al., 2011; Raaschou-Nielsen et al., 2010), were thus excluded from the asthma analysis, but are included in a separate section on lower respiratory tract symptoms in infants and toddlers.

EPA also considered issues regarding the timing of the exposure with respect to the specific outcome under study.

- In the only study of incident asthma (Smedje and Norback, 2001), measurements of formaldehyde in classrooms were taken at baseline and again two years later; the end of the follow-up period was two years after this measurement (4-year total follow-up). EPA considered this protocol to reflect a relevant exposure period.

- For studies of prevalence of current asthma (based on symptoms and medication use over the past year), EPA looked for information that supported the suitability of the exposure measure as a characterization of exposure during this time period. Examples include a study that collected exposure measures in at least two seasons or that examined season in the analysis.

- EPA considered exposure measures taken concurrently with completion of the asthma questionnaire to reflect a relevant exposure period for studies of asthma control (symptoms and medication use over the past 2–4 weeks).

- For results pertaining specifically to nighttime symptoms, EPA considered exposure measures taken in the home to provide a more relevant exposure measure than school-based exposures.

Exposure assessment

Based on the review of exposure assessments in the studies (see the general criteria for Exposure Assessments for Epidemiological Studies, Appendix A.5.1), EPA made the following decisions:

- EPA had lower confidence in the exposure measurements in two studies that used relatively short sampling periods (30 minutes and two hours, respectively, in 30 minutes and two hours, respectively, in Dannemiller et al., 2013; Hsu et al., 2012) and two studies in which the sampling time was not specified (Zhai et al., 2013; Choi et al., 2009). (Neither of these two authors responded to an email inquiry from EPA regarding this question.) Each of these four studies did contain some information regarding the specifics of the sampling protocol or quality control procedures and encompassed a wide range of exposures.

- Although Hwang et al. (2011) reported a geometric mean, this study did not provide more complete information on distribution of exposure levels (e.g., 75th percentile, or maximum value); thus, EPA also had lower confidence in the exposure description of this study.

- EPA also had lower confidence in the exposure measures of the study by Tavernier et al. (2006). This study used a 7-day measurement period in two locations in the home, and reported results by tertile of exposure. However, no information on the distribution of exposure levels (e.g., cutpoints for the tertiles) was provided, so it is difficult to interpret the results. The corresponding author did not respond to an email inquiry from EPA regarding this information. [The paper by Gee et al. (2005) appears to be the same study; this paper reported median levels of 0.03 and 0.04 ppm (0.037 and 0.049 mg/m^3) in the living room and bedroom samples.]

There was also variation in the exposure measurements used within the five occupational studies identified in this search (Neghab et al., 2011; Fransman et al., 2003; Herbert et al., 1994; Malaka and Kodama, 1990; Holness and Nethercott, 1989), with exposure assessments based on

one or more area samples in specific task areas, personal samples, or a combination of both. For hazard identification, an accurate characterization of "high" versus "low" exposure or "exposed" versus "nonexposed" may be able to provide a sufficient contrast to examine associations, even if there is considerable heterogeneity within the high exposure group. EPA considered the exposure assessment in each of these five studies to be adequate for this purpose, but noted the relatively high exposure [up to 0.08 mg/m^3 in the "low" exposure group of the Fransman et al. (2003)] would potentially result in an attenuated effect estimate.

Assessment of participant selection

The process through which study participants are identified, recruited, and selected, in addition to the participation rate, are important considerations in epidemiology studies. A selection bias can be introduced if both the exposure and the outcome (disease status) is directly or indirectly related to likelihood of participation. For the general population studies, EPA made the following decisions:

- EPA had high confidence in recruitment strategies based on geographic-based or population-based sampling frames (e.g., of residences or schools). However, EPA had lower confidence for the studies with this design that also had very low participation rates [(<20%) (Hsu et al., 2012; Billionnet et al., 2011; Hwang et al., 2011; Matsunaga et al., 2008)].

- EPA also had lower confidence in clinic-based, case-control studies that did not report any details of the recruitment of selection process (Choi et al., 2009; Rumchev et al., 2002), and in case-control designs that were not drawn from a defined population (Garrett et al., 1999a, b).

- EPA had low confidence in the selection process in the case-control study by Tavernier et al. (2006). Although cases and controls were drawn from two primary care practices, 95 cases were excluded because no age- and sex- matched control was identified.

A primary consideration regarding participant selection in the occupational exposure studies was the recruitment of current workers, that is, workers who remained in a workplace for some time (e.g., 2 or more years). This type of design could result in the "healthy worker effect," resulting in the potential loss of affected individuals from the workforce. EPA noted this as a limitation in all of the occupational studies. The participation rate in one of these studies was 66% (Fransman et al., 2003) and ranged from 87–100% in the other four studies. EPA did not consider this difference to be sufficient to conclude that the protocols should be viewed with different levels of confidence.

Assessment of potential confounding and other analysis issues

EPA approached the evaluation of potential confounding by considering critically important risk factors that could also be related to formaldehyde exposure (and are not in the causal pathway). Age and sex were considered key demographic variables, although it is not likely either

is associated with variability in indoor formaldehyde levels. EPA also examined information on potential correlation between formaldehyde and other air pollutants associated with allergy or asthma; the specific measures differed depending on the setting. The evaluation of the control for confounding was not based on whether a particular variable was or was not included in a model; rather a broader array of information was used, including the approach to modeling and information on patterns of exposure in the specific study population.

Based on these considerations, EPA made the following decisions:

- EPA had low confidence in three studies because of evidence of confounding that could not be addressed (Yeatts et al., 2012; Choi et al., 2009; Smedje et al., 1997; Norback et al., 1995). Two of these studies could not distinguish between effects of formaldehyde and effects of other exposures strongly correlated with formaldehyde (Yeatts et al., 2012; Smedje et al., 1997; Norback et al., 1995), and the third (Choi et al., 2009) did not address risk factors for the outcomes that were shown to vary between cases and controls, and that could reasonably be postulated to also be related to formaldehyde levels.

Reasons for different ratings within a study

- In some cases, different evaluation ratings were given for the different outcomes or analyses included a study:

  For Palczynski et al. (1999), the difference in evaluation ratings for children and adults for the skin prick test analyses is based on greater uncertainty regarding the timing of the exposure measure in this outcome in these two groups.

  For Garrett et al. (1999a, b), the inclusion of approximately 30% of the controls from the same household as the asthma cases and the inability to distinguish between ever- and current asthma resulted in a low confidence rating for the asthma analysis and a medium confidence rating for the skin prick test analysis.

  For Fransman et al. (2003), the ratings for allergies (low confidence) differed from that of asthma (medium confidence), due to the uncertainty regarding the specificity of the questions used to ascertain allergy history.

  For Herbert et al. (1994), uncertainty about time window of exposure measurement with respect to skin prick test results resulted in a "low" confidence rating for that analysis and a "medium" confidence rating for the asthma analysis.

Summary of reclassification of studies

This evaluation process resulted in the refinement of the inclusion criteria for asthma: the eligible population for asthma was changed from "humans" to "humans, age ≥4 years" because the respiratory disorder occurring in infants and toddlers may be related to, but is distinct from, asthma, which is more reliably diagnosed in school-aged children. As noted previously, four studies that had been identified as asthma studies were thus reclassified as studies of "lower respiratory tract symptoms in infants and toddlers." These studies, and the reasons for this reclassification, are:

- Raaschou-Nielsen et al. (2010)—limited to infants; outcome = wheezing episodes
- Roda et al. (2011)—limited to infants; outcome = lower respiratory tract infection (with and without wheeze episode)
- Rumchev et al. (2002)—limited to ages 6–36 months; outcome = asthma based on emergency room discharge data

Considerations of alternative classifications

This evaluation process necessarily results in the categorization of what is essentially a continuous measure (confidence level). In some cases, different overall confidence levels could be supported, depending on the emphasis that was placed on different strengths and limitations. In these situations, EPA considered the impact of alternative classifications. For examples, Smedje and Norback (2001) is the only study that examined incidence of allergies or asthma; the prospective design is a considerable strength of the study. However, the exposure assessment (conducted in classrooms in the baseline year and in Year 3 of the four-year follow-up) was limited by a high prevalence of values below the detection limit (54% of 1993 samples and 24% of 1997 samples were below 0.005 mg/m^3; geometric mean 0.004 and mean 0.008 mg/m^3), resulting in uncertainties in interpreting the analysis conducted using formaldehyde as a continuous measure. EPA classified this as a low confidence study because of the analysis, but also conducted a sensitivity analysis using an alternative classification of medium confidence.

Summary of overall evaluation of confidence

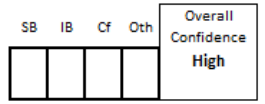
Based on the considerations described above, EPA developed an overall evaluation of its confidence in each study (or a specific analysis within a study), with high, medium, and low confidence categories. Table A-50 describes the criteria used in this classification. Because the exposure assessment was a primary consideration in this evaluation, it is presented as a separate column, with other aspects of study design and analysis combined in another column. The subsequent table in this section provides the more detailed documentation of the evaluation of observational epidemiology (see Table A-51); studies are arranged alphabetically within this table.

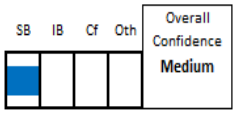
Table A-50. Criteria used to assess epidemiologic studies of respiratory and immune-mediated conditions, including allergies and asthma, for hazard assessment

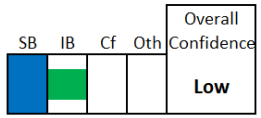
Overall evaluation	Exposure assessment	Study design and analysis
High confidence	**General population:** Exposure measure based on at least 3-d sample, corresponding to appropriate time window (e.g., measures in more than one season if time window covers 12 mos, or addressed season in the analysis. For inferences above 0.050 mg/m^3, exposure range includes large enough sample above	High specificity of outcome ascertainment; participant selection based on population-based sampling frame with high participation rate; confounding considered and addressed in design or analysis; analysis allows for examination of variation in effect in relation to variation in exposure level using analytic

Overall evaluation	Exposure assessment	Study design and analysis
	0.050 mg/m^3 to allow for meaningful analysis in this range. Work settings: Ability to differentiate between exposed and unexposed, or between low and high exposure.	procedures that are suitable for the type of data. Large sample size (number of cases)
Medium confidence	**General population:** More limited exposure assessment, or uncertainty regarding correspondence between measured levels and levels in the etiologically relevant time window. **Work settings:** Referent group may be exposed to formaldehyde or to other exposures affecting respiratory conditions (potentially leading to attenuated risk estimates)	Uncertainty regarding specificity of outcome ascertainment or participant recruitment process; confounding considered and addressed in design or analysis but some questions regarding degree of correlation between formaldehyde and other exposures may remain. Total sample size adequate but limited in stratified analyses.
Low confidence	**General population:** Short (<1 d) exposure measurement period without discussion of protocol and quality control assessment.	Low specificity of outcome ascertainment; high likelihood of confounding that makes it unable to differentiate effect of formaldehyde from effect of other exposure(s), limited data analysis (or analysis that is not appropriate for the data) or small sample size (number of cases)
Excluded (not informative)	Exposure range does not allow meaningful analysis of risks above 0.010 mg/m^3	

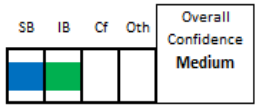
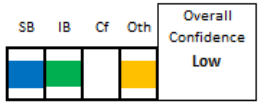
Table A-51. Evaluation of allergy and asthma studies

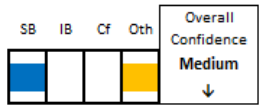
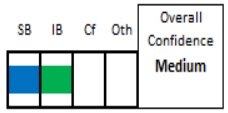
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Annesi-Maesano et al. (2012) (France) Schools: children (prevalence survey)	Schools randomly selected from defined geographic area, ages 9–10 yrs. Participation rate 81% in initial survey, 69% with full protocol.	5-d samples in classrooms; sampling from 108 schools; all classes of specified grade level per school. Median (75th percentile) 0.027 (0.034) mg/m^3 (estimated from figure). Protocol discussed.	ISAAC questionnaire Allergy: "sneezing and runny nose accompanied by itchy eyes out of cold in the past year" Asthma: asthma in past year (wheezing or whistling in the chest or wheezing or whistling in the chest at night-time or taken asthma treatment in the past year) Exercise induced asthma based on response to pulmonary function testing after exercise protocol. Exposure measurement blinded to outcome classification	Adjusted for age, gender, passive smoking, and paternal or maternal history of asthma and allergic diseases. Also examined dampness, gas appliances, ethnicity, socioeconomic status, and season. Other measures included: NO_x, $PM_{2.5}$, acetaldehyde, acrolein	Generalized estimating equation modeling, accounting for nonindependence of observations within-area (schools) environment, including climate. OR (95% CI) (CI estimated from figure). Models took into account within city correlations among participants. Additional stratification of asthma analysis by atopy status. Sensitivity analysis: exercise induced asthma limited to measures in same week (n = 4,643)	6,683	**Allergy (rhinoconjunctivitis) and Asthma**  No other pollutants were associated with rhinoconjunctivitis. $PM_{2.5}$ and acrolein were associated with asthma.

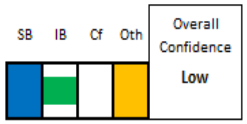
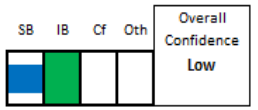
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Billionnet et al. (2011) (France) Residences: adults (prevalence survey) October 2003–December 2005	Nationally representative sample of residences (Indoor Air Quality Observatory study); 13.6% participation rate (567 of 4,165 households). Low participation rate	1-wk sample in bedroom; Median, 75th percentile (minimum, maximum) 0.0194, 0.028 (0.013, 0.0863) mg/m^3. Protocol discussed.	ISAAC questionnaire: Rhinitis based on self-report of, in the past 12 mos, sneezing, running or blocked nose without cold or respiratory infection. ECRHS: Asthma based on one of following criteria: (i) having an asthma attack in the last 12 mos; (ii) having been woken by an attack of shortness of breath in the last 12 mos; and (iii) currently using asthma medicine. Exposure measurement blinded to outcome classification	Covariates chosen if associated with asthma or rhinitis and affecting one or more effect estimates for volatile organic compound exposure measures by 20% or more. Adjusted for age, gender, smoking, education, relative humidity, time of survey, pets, mold, outdoor pollution sources within 500 meters. Did not specifically address correlation between formaldehyde and other exposures (other than noting that these were not among the higher correlations seen).	Generalized estimating equation modeling, accounting for nonindependence of within-area (dwellings) observations. OR (95% CI) (estimated from figure). Additional models took into account within dwelling correlations among participants. Compared nonparticipants (pollutant measures but no health questionnaire) and participants. Sensitivity analysis excluding relatives.	1,012	**Allergy (rhinitis) and asthma**  Low participation rate but potential for diffential participation (by formaldehyde exposure and disease status) unlikely.
Branco et al. (2020) (Portugul)	A total of 1,530 preschoolers (n=648 3–5 yrs) and primary	Daily exposure based on time-averaged air concentration	The ISAAC questionnaire was completed by parents or guardians, which	Potential confounders selected based on previous	Multivariate logistic regression for each individual	N = 1,530	**Wheezing** Not informative Analyses included ages 3–10 yrs of age

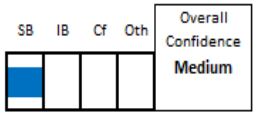
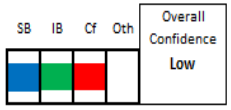
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
School: children (prevalence survey) 2013 - 2016	school children (n=882 6–10 yrs) were randomly recruited from urban and rural nursery (n=17) and primary schools (n=8) participating in the INAIRCHILD project. There were two phases in 2013/2014 and 2015/2016. Children < 3 yrs were excluded. Participants represented 39% of the original sample. No comparisons of participants and nonparticipants. 42% were aged 3–5 yrs, with less specific asthma diagnosis. Low participation raises concern for selection bias. PFT was only conducted in the 49% who reported wheezing or asthma diagnosis possibly introducing bias in	and reported time in specific school locations. Continuous monitoring in each room (24 hr to 9 d) (Branco et al., 2019). Time-activity obtained from parents' 24-hour daily diary, class timetables and teachers. Inhaled daily dose estimated using time-averaged exposure, inhalation rate for each activity and body weight. Mean HCHO concentration (SD) 35.3 (43.1) $\mu g/m^3$);	were validated by physicians. Spirometry measurements were taken in participants identified as asthmatic from the questionnaire responses or reporting ever having one or more asthmatic symptoms (wheezing, dyspnea, or nocturnal cough with no upper respiratory infection) (of 763, missing or failed in 269). Spirometry before and after bronchodilator using ERS/ATS and Global Initiative for Asthma guidelines conducted by pediatric doctors with pulmonary specialization. Methods and QA described. Asthma diagnosed based on symptoms (≥ 1) and PFT results using GINA guidelines. Skin prick tests conducted on children with PFT results using several aeroallergens (n=341, missing or failed for 153). Outcomes: reported active wheezing in last 12 mos (relevant to	experience and included site (urban, rural), study phase, sex, age group, BMI and parental history of asthma. Also controlled for surrogates of home indoor exposure including mother's education, living with smoker. Other covariates for contact with farm animals during 1st year of life, pets at home in previous year &/or 1st year of life.	pollutant as continuous variable (per IQR) or dichotomized using median, or regulatory cutoffs. Models also for all pollutants simultaneously.		**Asthma diagnosis**  Concern regarding potential for selection bias (low participation and missing values) and decreased specificity of asthma diagnosis by including very young children (< 5 yrs)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	PFT endpoints. Missing PFT data for 269 of 763 selected (35%).		pre-schoolers); reported asthma (does child have or ever had asthma?); diagnosed asthma by study physicians, FEV1/FVC <0.90, reduced FEV1 (<80% predicted), asthma diagnosed in 5.5%, asthma with or without aeroallergen sensitization, and no asthma. (Inclusion of notable proportion of children aged <5 yrs likely decreased specificity of asthma diagnosis.				
Choi et al. (2009) (Korea) Residences: children (and adults?) (case-control study) March–June 2006	Conducted in university outpatient clinic; recruitment procedure for cases or controls not described. Mean age cases 15.4 yrs (SD = 3.4; controls 16.2 yrs (SD = 4.1)	Household sample in living room at location away from sources of VOCs (sampling period not reported, but closed windows, no smoking or use of potential sources, and use of duplicates). Geometric mean 0.043 mg/m^3, 75th	Atopic dermatitis and allergic asthma: based on medical history, skin prick test and IgE (criteria not provided)	No information on socioeconomic status; higher percentage of cases lived near roads or in industrial area (21%, 34%, 44% of controls, dermatitis, and asthma cases, respectively). Housing age <3 yrs old in 29%, 40%, and 58% in controls, dermatitis, and asthma cases,	Nonparametric (Mann-Whitney) comparison of formaldehyde by group; geometric mean, 25th, and 75th percentiles reported.	50 atopic dermatitis cases, 36 asthma cases, 28 controls	**Allergy (atopic dermatitis) and lower respiratory tract symptoms in infants and toddlers**  Selection and recruitment process not reported; sampling period not reported and specific criteria for case definition not reported; potential confounders (age and type of housing and location differed between

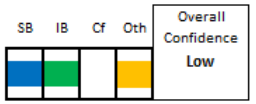
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		percentile 0.115 mg/m^3.		respectively; and 50%, 56%, and 72% of controls, dermatitis cases and asthma cases lived in apartments.			cases and controls, as measure of socioeconomic status) not addressed. Limited analysis.
Dannemiller et al. (2013) (United States) Residences: children (asthma control) July 2008–February 2010 **Related reference:** Sandel et al. (2014)	Low-income homes in Boston, recruited from past allergy cohorts, asthma clinics, newspaper ads, and referrals from other participants. (Boston Allergen Sampling Study). 79% (37 out of 47) participated in this analysis. Mean age 10.5 yrs. Boston Allergen Sampling Study.	30-minute pumped air sample in kitchen. Median 0.044 mg/m^3; 31% >0.060 mg/m^3; maximum = 0.162 mg/m^3. Protocol discussed; analysis of sources of exposure	Asthma control (5 questions) [based on validated questionnaire]; symptoms and inhaler use in past 4 wks	Examined season, temperature, and relative humidity *(email from Karen Dannemiller to Glinda Cooper, May 6, 2015)*	Log_{10}-transformed formaldehyde; *t*-tests.	37 asthma cases (out of 47 children in study, 79%)	**Asthma control**  Recruitment was not from a well-defined population. Limited exposure measurement period (but quality control details provided).
Fransman et al. (2003) (New Zealand) Wood workers (prevalence survey)	Plywood mill workers, participation rate 66%. Internal comparison by exposure level. Mean duration 4.7 yrs in mill, 2.7 yrs in current job. Workers' knowledge of	Personal samples (15-min samples); above 0.100 (geometric mean 0.260 mg/m^3). Limit of detection 0.030 mg/m^3.	Allergy symptoms: self-report of sensitivity to house dust, food, animals or grasses/plants. Asthma: Current asthma medication use; past 12 mos, asthma attack or being woken by shortness of breath	Adjusted for age, gender, ethnicity, and smoking for comparisons between high and low exposure within workplace. Weaker association seen with terpenes. Inhalable dust,	Logistic regression, OR (95% CI)	112	**Allergy (allergy symptoms)**  Uncertain impact of outcome classification and uncertainty regarding details of analysis; see

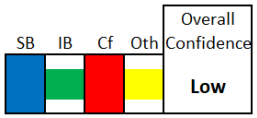
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	formaldehyde exposure not discussed.			abietic acid, and endotoxin also measured but not clear if these were considered in the analysis of the allergy symptoms data			asthma discussion for other limitations **Asthma**  Selection out of the exposed work force of "affecteds" possible in this type of prevalence study. "Low" exposure group exposed to levels of formaldehyde up to 0.080 mg/m^3. Either limitation would result in reduced (attenuated) effect estimate.
Garrett et al. (1999a, 1999b) (Australia) Residences: children (prevalence survey)	Combined analysis of cases and controls from a case-control study of asthma in two rural towns. Recruitment through schools and medical centers; additional advertisement for nonasthmatic children. 30 of the 95 controls were from same households as cases; the 65 other controls	4-day household samples (4 seasons), multiple locations; up to 0.139 mg/m^3. Protocol discussed. Separate paper about exposure measures. 74% of children had lived in same house for at least 5 yrs.	Allergy: 12 allergen skin prick test (cat, dog, grass mix #7, Bermuda grass, house dust, 2 dust mite, 5 fungi). Asthma Parent report of doctor-diagnosed asthma. Mean score 4.6 in asthma cases, 0.7 in controls on respiratory symptom questionnaire completed at last home visit (symptom frequency, 4 categories, over past year of: cough, cough in the	Adjusted for parental asthma history, sex; other factors examined but not needed in final model (passive smoke, pets, indoor NO_2, fungal spores, house dust mite allergens)	Prevalence (n, %) by exposure group; logistic regression, OR (95% CI); figure showing wheal size and number of positive responses by exposure group. Evaluated relation between formaldehyde and NO_x, house dust, fungal spores, housing age.	145 in allergy analysis; 53 cases, and 95 controls in asthma case-control analysis	**Allergy (skin prick tests)**  Uncertainty about about effect of recruitment process and about time window of exposure measurement with respect to skin prick test results. **Asthma**

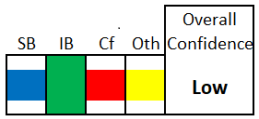
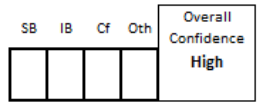
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	were from 37 households.		morning, shortness of breath, waking due to shortness of breath, wheeze/ whistling, asthma attacks, chest tightness, and chest tightness in the morning). Exposure measurement blinded to outcome classification.				 Uncertainty about asthma definition (current asthma or ever asthma?). Uncertainty about effect of recruitment process and ability to fully address household correlation of cases and controls; could result in attenuated effect estimate. Incomplete reporting of results (adjusted results reported as "not statistically significant")
Herbert et al. (1994) (Canada) Wood workers (prevalence survey) **Related reference:** Herbert et al. (1995)	Oriented strand board manufacturing, mean duration 5.1 years. Referent group = oil field workers, not exposed to gas or vapors, mean duration 10.0 years. Participation rate 98% in workers, 82% in comparison group. 99 exposed, 165 referents. Because both	Area samples. 21 hrs continuous sampling on two separate days); range 0.090 to 0.330 mg/m^3	Allergy: 6 allergen skin prick test (wheat, rye, Alternaria, cat, house dust, birch). Asthma: International Union Against Tuberculosis and Lung Disease (1986) questionnaire, described and validated in (Ravault and Kauffmann, 2001): (asthma; lower respiratory tract symptoms (list includes woken by shortness of	Adjusted for age and smoking; dust measured and reported as low, not included in analysis	Logistic regression, OR (95% CI); prevalence of "outcome" (positive responders) not reported	99 exposed; 165 referents	**Allergy (skin prick tests)**  Uncertainty about time window of exposure measurement with respect to skin prick test results; some uncertainty about referent group. **Asthma**

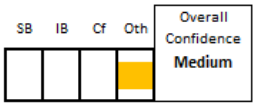
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	groups are "exposed" workers, healthy worker effect unlikely. Some uncertainty about effect of exposures in the referent group		breath; attacks of wheeze, wheeze with chest tightness.) [increased prevalence of lower respiratory tract symptoms associated with lower FEV_1 or FEV_1/FVC in these workers]. Time frame of asthma definition interpreted to be relevant to occupational exposure. Exposure measurement blinded to outcome classification				 Selection out of the exposed work force of "affecteds" possible in this type of prevalence study, and some uncertainty about referent group.
Holness and Nethercott (1989) (Canada) Funeral home workers (prevalence survey)	Participants recruited from list of funeral homes, 86.6% participation; 79.8% of embalmers were active embalmers (healthy workers); community referent (service organization and students)—potential differences (weight, smoking)	2 area samples (impingers), during embalming, 30 to 180 min. Range in exposed 0.10–1.0 mg/m^3, referent mean 0.025 mg/m^3; adequate exposure contrast likely for comparison of exposed and referent.	American Thoracic Society (Ferris, 1978) questionnaire: wheeze (no details of questions)	Univariate analysis; did not consider other variables	Frequency by group and *p*-value from a logistic regression	N=84 exposed; N=38 referents	 Uncertainty regarding asthma definition. Selection out of the exposed work force of "affecteds" possible in this type of prevalence study; would result in reduced (attenuated) effect estimate. No consideration of potential confounding
Hsu et al. (2012)	Initially recruited through randomly selected kindergartens and	2-hr household sample (probably	Initial screening through parent report of history of 2 or more diseases (asthma,	None addressed in analysis. Similar season distribution in	Mann-Whitney U test for case-control differences in	48 allergic rhinitis, 36 eczema, 9 asthma	**Allergy (rhinitis, eczema) and asthma**

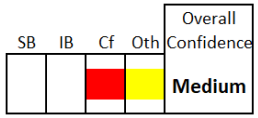
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
(Taiwan) Residences: children (case-control) August 2008–September 2009	day care centers; 73% of successfully contacted agreed to send questionnaires to families and 68% of the questionnaires were completed. Selected for follow-up if had not moved or renovated house since birth. Of the 980 potential cases and 802 potential controls selected, 267 (27%) and 89 (11%) participated in clinical exam; 59 cases and 42 controls (22% and 47% of cases and controls, respectively, completing exam) also completed home exposure measures.	bedroom); Median 0.076 mg/m^3; 75^{th} percentile 0.030 mg/m^3. Limited sampling period with no information on protocol.	allergic rhinitis) or symptoms (wheezing, coughing at night, eczema, sneezing, runny or stuffy nose) during last 12 months; confirmation of asthma, rhinitis, and eczema by clinical examination. Controls answered "no" to all of the disease and symptom questions. Exposure measurement blinded to outcome classification	cases and controls	exposure distribution. Median, 25^{th} and 75^{th} percentiles given for cases and controls. *P*-values reported if <0.10. No additional modeling of the formaldehyde data undertaken.	cases, and 42 controls	 Low and differential (at various steps) participation rate. Short exposure sampling period and no information on protocol. Limited analysis. Uncertainty regarding distribution (% <LOD). In addition, small sample size (*n* = 9) for asthma.
Hulin et al. (2010) (France) Residences: children	Two samples: 1) urban area, French Six Cities Study (ISAAC). Random selection of 18	7-d sample in living room. Protocol discussed. Median 0.019 mg/m^3,	Ever asthma and current asthma (parent report of use of asthma medications or wheezing in past 12 mos).	Adjusted for age, sex, family history of allergy, passive smoke exposure during childhood,	OR (95% CI) by above and below median. Also analyzed by stratified by	Urban: (32 cases, 31 controls). Rural: (24 cases, 27 controls).	**Asthma** 

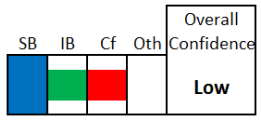
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
(case-control)	schools; nested case-control study of asthma 2) Rural area; nested case-control study of asthma (FERMA) (rural sampling fro regular contact with farm animals) Examined nonparticipants	maximum 0.075 mg/m^3	Exposure measurement blinded to outcome classification	allergic rhinitis, and season. Considered nonindependence of participants in similar neighborhood. Assessed collinearity with other measures (NO_x, $PM_{2.5}$)	location (urban, rural)	Combined: 56 cases, 58 controls (but 9 rural and 7 urban excluded, unspecified number excluded from analysis limited to current asthma	Small sample size and uncertain interpretation of the stratified analyses (and unspecified n in analysis of current asthma).
Hwang et al. (2011) (Korea) Residences: children (case-control) May 2008	Case-control study, drawn from 1,005 elementary students (one school, all grades) (84% participation rate). 33 cases (out of 129?) and 40 controls (out of unspecified number) agreed to participate in environmental measurement study. Controls selected from respondents with no asthma symptoms or diagnosis, age and sex matched to cases.	3-day household sample (2 rooms) and personal sample. Geometric mean, controls: 0.036 mg/m^3 (no information on upper distribution reported).	Self-report asthma symptoms or physician-diagnosed asthma based on ISAAC questionnaire	Adjusted for age, gender, income, parents' education, passive smoking	Log-transformed; logistic regression, OR (95% CI)	33 cases, 40 controls	**Asthma**  Asthma definition does not distinguish between current asthma and ever asthma. Uncertainty regarding selection processes [high prevalence of family history of asthma in cases (86%) and controls (96%)]; uncertainty about analysis and distribution

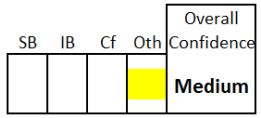
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Huang et al. (2017) (Shanghai, China) Residences: children (case-control) March 2013-December 2014	Participants in a previous cross-sectional study (2011-2012) selected from 88 kindergartens located in 6 Shanghai districts (note: references for cross-sectional study stated 72 kindergartens selected in 5 districts, N = 14,884). Included if homes were not renovated in the previous 2 years and agreed to an on-site home inspection, N=454 residences, 4.5% of cross-sectional survey for 10,182 participants with contact information (409 of 454 residences assessed), 5 - 10 years old. Concern for selection bias since eligibility was based on ever asthma status and home renovation.	Continuous formaldehyde sampling in child's bedroom, 24 hours, in breathing zone (detection range: 0.012-0.08 mg/m^3). Monitors calibrated before sampling. Average concentration (µg/m^3), 24-hr 21.5 ± 13; 6-hr 22.2 ± 17.9 Range 6.0 – 60.0 µg/m^3, with 2 bedrooms higher Short sampling duration less likely to represent concentrations over the previous year	History of airway diseases using translated ISAAC questionnaire; cases responded "yes" to symptom/disease question in either phase (cross-sectional or case-control phases) from questionnaire. Current rhinitis: In the past 12 months, has your child had a problem with sneezing, or a runny, or a blocked nose when he/she did not have a cold or the flu?	Covariates considered in models based on literature and previous analyses, included age, sex, family history of atopy, family annual income level, household ETS, household dampness-related exposures, antibiotics exposure during 1st year of life, home decoration around time of birth, season of sampling. Higher proportion of homes with mechanical ventilation among current rhinitis cases compared to controls (77.5% versus 65%)	Differences between cases and controls compared using Kolmogorov-Smirnov test. Multiple logistic regression models per IQR increment or quartile of formaldehyde concentration.	N = 409	**Current rhinitis**  Concern for selection bias, difference in ventilation methods by case status suggests uncontrolled confounding, Low formaldehyde concentrations

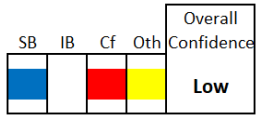
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Isa et al. (2020a) (Malaysia) Schools: children (prevalence survey) August-November 2018 & February 2019	8 randomly selected schools in Hulu Langat, Selangor, Malaysia, randomly selected students from 4 classes (Form two, aged 14 years). Excluded students reporting smoking in last 12 months or treated with antibiotics in last 4 weeks. Participation not reported.	Formaldehyde concentrations measured during class time using PPM Formaldemeter (accuracy of 10% at 2 ppm). Monitors 1 meter from ground in center, 4 one-hour periods. Concentration (reported as mg/m^3, but appears to have been µg/m^3) median (IQR) Urban 13.2 (9.3); Suburban 3.1 (5.2) Uncertainty in concentrations given short sampling duration	Asthma & allergy information and symptoms within defined period using ECRHS and ISAAC questionnaires. Responses were blind to environmental data. Allergy skin prick test for mites, fungi and cat allergens after 15 minutes measuring wheal diameter (atopy defined as ≥ 3 mm). Respiratory symptoms in last 12 months: wheezing, daytime breathlessness, nocturnal attacks of breathlessness. Allergic symptoms in last 12 months: rhinitis, skin allergy.	Regression models controlled for atopy, sex, doctor's diagnosed asthma, parental asthma/ allergic and location of schools. No adjustment for ETS. Associations also observed for NO_2 – unknown impact of confounding on formaldehyde associations.	2-level hierarchic multiple logistic regression, OR (95% CI). Concerns for choice of exposure metric (continuous variable) with no information about distribution below the LOD.	N=470	**Allergy (rhinitis, dermal, skin prick tests)**  **Low** Uncertainty in exposure concentrations and distribution given short sampling duration, very low concentrations in half the schools with unclear proportion of samples less than the LOD, and analysis using concentration as a continuous variable. Participation details not reported.
Kim et al. (2011) (Korea) Schools: children (prevalence survey)	12 schools, 2-3 randomly selected classrooms per school Participation rate 96%; 450 excluded based on missing data)	7-day samples in classrooms. 1 SD above mean = 36 µg/m^3; maximum = 47 µg/m^3. Protocol	Current medication use or had asthma attack in past 12 months. Exposure measurement blinded to outcome classification	Adjusted for age, sex, self-reported pet or pollen allergy, environmental tobacco smoke at home, other home	Logistic regression, OR (95% CI) per 10 µg/m^3 increase; additional modeling to account for within school and	2,365	**Asthma** 

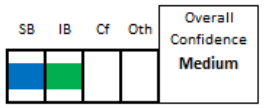
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
November–December 2004		discussed, closed windows.		environment (indoor dampness, remodeling, changing floor, age of home). All samples within same season.	within city correlations.		
Krzyzanowski et al. (1990) (United States, Arizona) Residences: adults, children (prevalence survey) **Related references:** Quackenboss et al. (1989a); Quackenboss et al. (1989b)	Selected from 202 households (stratified sample from municipal employees). 2,322 completed baseline survey; subgroups selected based on housing characteristics (type, age, remodeling). Clusters within similar outdoor PM and pollen levels. Participation rate not reported but sampled nonresponders: higher proportion of current smokers among refusals (35% versus 27%)	Two one-week household samples (different seasons), multiple locations; Mean 0.032 mg/m^3; maximum 0.172 mg/m^3 (most <0.074, only a few above 0.110 mg/m^3) Protocol discussed (separate paper).	Asthma: American Thoracic Society (Ferris, 1978) questionnaire; doctor-diagnosed asthma (ever and current) and symptom questions: wheezing apart from colds, 2 or more attacks of shortness of breath with wheezing in last year. Exposure measurement blinded to outcome classification	Environmental tobacco smoke. Also examined NO_2	Contingency tables, stratified by age group and for children, by environmental tobacco smoke exposure.	Adults: 613 Children: 298	**Asthma, children and adults**  For children, relatively small # in higher exposure categories. For adults, incomplete reporting of results.
Lajoie et al. (2014)	Asthmatic children with exacerbation requiring medical	Pre and post-intervention. Passive air	Variable number with complete data for each outcome. Participants	Potential confounders for asthma outcomes	Power calculation reported. Multivariate	For ISAAC questionnai re,	**Current asthma symptoms**

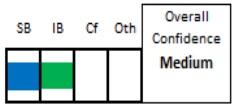
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
(Quebec, Canada) Intervention study October 2008 – June 2011	care in the past year referred by physicians at tertiary care center, 3 – 12 years old, (n=83, 71.5% of those meeting inclusion criteria) in homes with low ventilation rates (<0.30 ACH). Randomly assigned to intervention to increase ventilation rates by 0.15 ACH (n=43) and control (n=40).	sampling for formaldehyde in bedroom, 6-8 days, during winter and summer seasons. Other measurements for $N0_2$, VOCs, dust, house dust mites, cat and dog allergens, airborne mold spores	were not blinded, although technicians were. Formaldehyde-specific Intervention/Control Proportion with ≥ 1 episode of wheezing over last 12 months, ISAAC questionnaire administered to parents: 43/39; Mean number of days with asthma symptoms per 14 day period (≥ 1 coughing, wheezing, chest tightness, disturbed sleep or trouble breathing Symptoms diary: 37/32; administered to parents 2 weeks per month from November – March in 2010 and 2011; Asthma control over one month, Asthma quiz: 31/25;	were age, gender, parents' level of education, and eczema. Comparing baseline concentrations formaldehyde, NO_2, and dust mites were comparable, Toluene and mold spores were higher in intervention group. Comparing year 1 to year 2, reductions in formaldehyde, toluene, styrene, limonene, and alpha-pinene, airborne mold spore concentrations were significantly different for intervention group compared to control. NO_2 concentrations increased. Allergens in mattress and rugs	linear models Formaldehyde analyses used results in intervention group only. Change from year 1 to year 2 in prevalence of asthma symptoms and medical care in the past year associated with a 50% reduction in formaldehyde concentration analyzed using mixed liner models with repeated measures	interventio n n = 43, control = 39	 **Medium confidence** Small sample size Other coexposures that have been associated with asthma symptoms also declined in intervention group (toluene, ethylbenzene, styrene, limonene, alpha-pinene, airborne mold spores, although formaldehyde reduction was greatest.

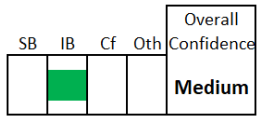
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
				in bedroom did not change.			
Li et al. (2019) (Hong Kong) Birth cohort September 2013 to April 2014	Infants aged < 4 months attending 14 maternal and child health clinics between September 2013 to April 2014, stratified by family history of asthma, family history of allergy and no family history. Included if locally born ethnic Chinese, age ≤ 4 months, Birth weight ≥ 2.5 kg, gestation ≥ 36 weeks, cared for at home, telephone numbers available, mothers aged ≥ 18 years, Cantonese speaking. Excluded if congenital disease, cared for at child-care center > 20 hours/week, moving after recruitment. Of 14,755 eligible, 4310 agreed to	Air sampling (NO_2, formaldehyde) using standardized diffusion samplers at 6 months of age. NO_2 10 – 14 day sampling period. Formaldehyde 72 hour sampling period using ISO 16000-4 method. Concentrations not reported.	Baseline information obtained using validated ISAAC questionnaire completed by parents prior to age 4 months. Weekly respiratory health diary and monthly health telephone survey blinded to exposure status until 18 months of age. New onset wheeze (time to event) measured from 6 to 18 months of age. 120 (12.5%) infants had new onset wheeze at an average of 13.2 months.	Potential confounders selected from baseline characteristics associated with formaldehyde concentrations using log-rank test, $p < 0.25$. Stepwise adjustment, final models adjusted for NO_2, sex, neonatal respiratory illness, having a sibling, family history allergy or asthma, pets, or cooking fuel. No control for smoking or ETS.	Cox regression in entire sample; formaldehyde modeling as continuous variable	N = 963	**Time to onset of wheeze event**  **Low** Concern for selection bias. Participation rate was very low (29% of eligible agreed) and of those selected there was notable data loss, data was complete for 67%. No comparisons of participants and nonparticipants and no descriptive statistics provided for study sample. No control for smoking or ETS.

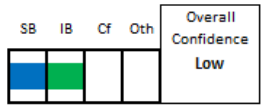
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	participate (29%). After stratification by family history, 1434 were recruited and data were complete for 963. 471 subjects had been lost because of invalid outcome or air samples or they dropped out. No comparisons of participants with nonparticipants. No descriptive statistics provided for study sample.						
Liu et al. (2018a) (China) Hospital based case-control: children September 2016 to March 2017	Recruited 180 children with an asthma diagnosis from hospital and 180 healthy controls in same city (Changchun) during September 2016 to March 2017. Administered ISAAC questionnaire, validated for children in Korea. Asthma severity assessed with pulmonary function tests.	Indoor area samplers placed 1 - 1.5 meters above ground, doors and windows closed 12 hours prior. HCHO sampled in living room and bedroom with QC-2B sampler, Beijing Municipal Institute of Labor Protection method.	Asthma diagnosis via ISAAC responses (2 or more incidents of cough, wheezing, and dyspnea for 3 or more consecutive days). In addition, FEV_1 increased by >15% after β-agonist inhalation and persistent asthma was stable for 3 or more months prior to study.	History of allergy, breast feeding, ETS and indoor plants were associated with asthma status. Included in model with $PM_{2.5}$ and HCHO. Sex, mean age, mean BMI and race were comparable between cases and controls.	Associations with pollutant concentration (quartiles) analyzed with multivariate regression.	180 cases; 180 controls	**Current asthma symptoms**  **Medium** While reporting details were brief, citations were given and appropriate methods for exposure and outcome ascertainment appear to have been used and the sampling period for HCHO was adequate. Coexposures to PM and NO_2 were simultaneously controlled.

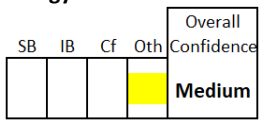
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	Children excluded if medical treatment with vitamins or antibiotics within 3 month, severe organ failure (heart, renal and other serious disorders).	Citation for method provided. Sampling period was 2 months. Median (range) µg/m^3 HCHO Asthma 38.35 (12.04 – 142.12) Control 25.11 (12.26 – 94.34) NO_2 and PM also measured.					
Madureira et al. (2016) (Porto, Portugal) Children, case-control, October 2012–April 2013	Random recruitment of 38 residences among asthmatic children and 30 residences among nonasthmatic children previously identified in a cross-sectional study (Madureira et al., 2015). Parents volunteered to respond to ISAAC questionnaire for n=1,099 children (aged 8–10 yrs, 69% of recruited).	Measurements of VOC, aldehydes, PM2.5, PM10, bacteria, fungi, carbon dioxide (CO2), temperature and relative humidity levels were conducted simultaneously both indoors and outdoors. Sampling and analysis methods described. Continuous passive	For asthma cases, parents responded yes to both of 2 questions in ISAAC questionnaire: 1) Has your child ever had asthma diagnosed by a doctor? and 2) In the past 12 mos, has your child had wheezing or whistling in the chest? Parents of controls responded no to both questions.	Higher proportion of cases were boys. Comparable for age, BMI and parental education level, family history of allergic disorders and number of siblings was slightly higher in cases. No other chemical or biological risk factors differed between groups (except limonene was higher in control). Analyses were not adjusted for	Concentrations (7-day means) compared between groups.	Cases n=38 Controls n=30	**Current Asthma**  **Low** Small sample size, potential for selection bias, no adjustment for confounding and some differences noted between cases and controls

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	Excluded respondents with a recent renovation or who had moved since responding. No information comparing participants to nonparticipants. Potential exists for selection bias with greater environmental controls among asthmatic families. Although extent of bias impact unknown, TVOCs, acetaldehyde and ventilation rates higher in control homes, but not PM or bacteria and fungi counts.	sampling for formaldehyde and other VOCs and aldehydes in bedroom over 7 d. Formaldehyde concentrations all above the detection limit.		potential confounders.			
Malaka and Kodama (1990) (Indonesia) Wood workers (prevalence survey)	Plywood mill workers, random sample of exposed workers (based on measurements), stratified by smoking, work duration (<, ≥ 5 yrs), (random sampling process not specified). Random sample of	Personal and area samples (duration not reported); above 200 (mean 910, up to 3,480 µg/m^3). Nonexposed areas based on measure-ments (e.g.,	American Thoracic Society (Ferris, 1978) questionnaire. Asthma defined as "Ever had attack of wheezing that made you feel short of breath?" or ever had asthma and if so, do you currently have asthma? A lso included	Adjusted for age, smoking, dust	Percent by exposure status, OR, *p*-value 95% CI not reported (but could be calculated for crude OR estimate)	93 exposed; 93 referents	**Asthma**  Selection out of the exposed work force of "affecteds" possible in this type of prevalence study. "Unexposed" exposure group exposed to levels of formaldehyde up to

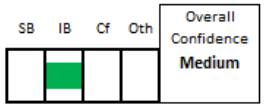
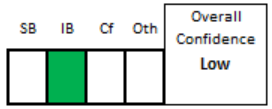
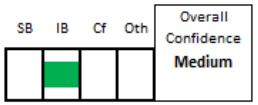
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	nonexposed (defined based on area measures and job history), matched to exposed by age, duration, and smoking. 93% participation rate and mean duration about 6 years in both groups.	warehouse, saw mill)	"occupational asthma" (not defined). Since purpose of study was the impact of occupational exposure, asthma definition is iinterpreted to be relevant to current status. [Increased prevalence of asthma associated with lower FEV_1 or FEV_1/FVC in these workers].				0.086mg/m^3. Either limitation would result in reduced (attenuated) effect estimate. "Occupational asthma" not defined and "ever" asthma may differ from current prevalence.
Matsunaga et al. (2008) (Japan). Residences: adults (Prevalence survey)	Pregnancy cohort, enrolled 2nd trimester. Recruited through pregnancy clinics and obstetrics departments. 17% of pregnant women in the city participated; recruitment extended to other areas. Low participation rate. Internal comparison group.	24-hour personal sample; 60th percentile 33 mg/m^3, 90th percentile 58 mg/m^3	Allergy: Self-report of medical treatment (medication use) for atopic eczema or allergic rhinitis in past 12 mos. Exposure measurement blinded to outcome classification. Asthma: Self-report of medical treatment (medication use) for asthma in past 12 mos.	Adjusted for age, gestation, parity, family history (of asthma, atopic eczema, allergic rhinitis), smoking status, current passive smoking at home and work, mold in kitchen, indoor domestic pets, dust mite antigen level, family income, education, and season of data collection. Also examined NO_2	Logistic regression, OR (95% CI) by 4 exposure categories (30th, 60th and 90th percentiles); also presented dichotomized at 90th percentile. Results also stratified by family history of allergies.	998 21 asthma cases, 57 eczema, 140 rhinitis cases	**Allergy (atopic eczema, rhinitis) and asthma**  Low participation rate but potential for diffential participation (by formaldehyde exposure and disease status) unlikely. For allergy, lack of data pertaining to sensitivity and specificity of these questions. Limited to one-day exposure sample (but did address season in analysis). For asthma, potential low sensitivity of outcome the questions, and in addition, small #

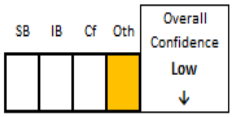
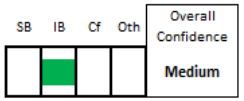
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Mi et al. (2006) (China) Schools: children (prevalence survey) November–December 2011	10 schools, 3 classrooms (7th grade) per school. Participation rate 99%	4-hour (school day) air samples; some information on measurement protocol. Minimum = 0.003 mg/m^3; (unclear if this is ½ of LOD?; 1 SD above mean = 18 µg/m^3; maximum = 20 µg/m^3.	ECRHS definition Medication use or asthma attack in past 12 months; additional questions on lower respiratory tract symptoms (in past 12 months, wheeze or whistling in the chest, daytime breathlessness attack at rest or after exercise, nighttime breathlessness attack). Exposure measurement blinded to outcome classification	Adjusted for age, gender, smoking, observed water leakage and indoor moulds. Also examined temperature, relative humidity, indoor CO_2, indoor O_3, and examined collinearity of exposures.	Logistic regression, OR (95% CI) per 0.010 mg/m^3 increase.	1,414	**Asthma**  Uncertainty about exposure distribution and analysis (e.g., percent <LOD and treatment in analysis as continuous variable)
Neamtiu et al. (2019) (Romania) Children: schools	Schools Indoor Pollution and Health: Observatory Network in Europe (SINPHONIE) project, 2010 to 2012. The authors analyzed the data for Romania, which included 5 primary schools in one county (2 rural, 3 urban), and 3 classrooms per school were selected. Questionnaire responses for October to December 2011	Formaldehyde measured in each classroom, 5 d sampling period. Passive samplers, Radiello cartridges, impregnated with 2,4-dinitrophenylhydrazine using ISO 16000-2 protocol. Analysis within 48 hrs using a validated method from European Commission.	Questionnaire responses on respiratory symptoms and allergic health conditions in the past week. Questions were taken from ISAAC and translated. Asthma-like symptoms defined as difficult breathing, dry cough and wheezing in the past week (any symptom Allergy-like symptoms defined as skin conditions (e.g., rash, itch, eczema), eye disorders (e.g., red, dry, swollen, itching, or burning eyes, or sensation of "sand in	Analyses controlled for age, sex, ETS in the past week, microclimate parameters (NO_2, CO, CO_2, temperature, relative humidity, ventilation rate.	Multivariate analysis of formaldehyde categorized as high (> 35 µg/m^3) and low (≤ 35 µg/m^3) based on the median.		**Asthma-like symptoms, Allergy-like symptoms**  **Medium** Selection of schools was part of a larger European framework. Appropriate methods for exposure assessment and outcome ascertainment instruments appear to have been used although endpoint, asthma-like symptoms, is not specific to current asthma definition.

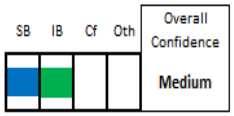
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	for 139 male and 141 female students; 89.7% response rate for children	Detection limit was 0.1 µg/m^3; median = 34.83 µg/m^3; maximum = 66.19 µg/m^3.	the eyes," and rhinitis symptoms (e.g., itching nose, sneezes, and/or stuffy or blocked Nose). Outcome definition (asthma-like symptoms) may have reduced specificity compared to definition for current asthma				Outcome definition for allergy-like symptoms using ISAAC questionnaire included combined symptoms of rhinitis (nose), eye and skin conditions.
Neghab et al. (2011) (Iran) Workers: melamine-formaldehyde resin plant (prevalence survey)	Exposed: melamine-formaldehyde resin plant workers. Referent group: office workers from same plant, no present or past exposure to formaldehyde or other respiratory irritant chemicals. Participation rate 100%. Duration ≥2 yrs	Area samples (40 min) in 7 workshops and 1 area sample in office area. Exposed (mean ± SD) 0.96 (±0.49) mg/m^3; unexposed = nondetectable.	American Thoracic Society (Ferris, 1978) questionnaire (modified): wheezing symptoms (no details of questions)	No covariates considered in the symptom analysis. Similar in demographics and current smoking (but smoking frequency higher among exposed)	Fisher's exact test, OR (*p*-value)	n = 70 exposed, 24 unexposed	**Asthma**  Uncertainty regarding asthma definition. Selection out of the exposed work force of "affecteds" possible in this type of prevalence study; would result in reduced (attenuated) effect estimate.

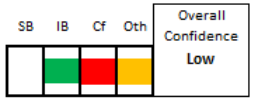
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Norback et al. (1995) (Sweden) Residences: adults (nested case-control)	64% participation rate for cases, 57% for controls	2-hr household sample (bedroom). Limited sampling period in closed residence with no point formaldehyde emissions; sampling and analytic protocols referenced Andersson et al. (1981), LOQ 0.1 mg/m3}; range reported as <0.005 to 0.110 mg/m^3, thus most were <LOQ)	Positive response to: asthma attack in past 12 mos, nocturnal breathlessness in past 12 mos, or current use of asthma medication. Controls answered no to all questions	Adjusted for age, sex, current smoking, wall-to-wall carpets, and house dust mites. Formaldehyde measure reported to be strongly correlated with total volatile organic compounds.	Log-transformed, logistic regression, OR (95% CI) per 0.001 mg/m^3 increase. Mean subtracted from each observation to reduce collinearity with VOCs	47 cases, 41 controls	**Asthma**  Uncertainty about exposure (most values <LOQ). Similar results for volatile organic compounds, and not possible to distinguish effects of formaldehyde and these other compounds; could result in inflated effect estimate.
Norbäck et al. (2017) (Malaysia) Schools: children 2007	8 randomly selected schools in Johor Bahru, Malaysia, randomly selected 15 students each from 4 randomly selected classes (Form two, aged 14 yrs). Participation 96%	Sampling and analytical methods were described. Formaldehyde sampled continuously over 7 d in each classroom using diffusion samplers. Samplers	Standardized questionnaire completed by students with parents blinded to environmental measurements. Rhinitis defined by two questions combined regarding nasal catarrh or nasal congestion. Cases defined by reporting symptoms	There were no significant correlations between CO_2, NO_2 or formaldehyde and any of the measured VOC. Models adjusted for other indoor chemical exposures,	Stepwise multiple logistic regression for symptoms including indoor exposures (CO_2, NO_2, formaldehyde and VOC by diffusion sampling and pumped air sampling),	N = 462	**Allergy**  **Medium** Quantitative results were not reported. Very low indoor formaldehyde concentrations

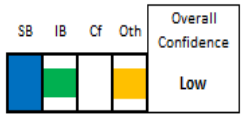
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		placed 2 meters above floor. Mean concentrations formaldehyde indoor 4.2 µg/m^3, max 18.0 µg/m^3, 100%>DL Outside 5.5 µg/m^3, max 6.0 µg/m^3, 100%>DL	weekly over a 3-mo period.	personal factors and home environment factors.	personal factors (sex, race, current smoking, atopy, parental asthma/allergy) and home environment factors (ETS, dampness/mold, recent indoor painting). 3-level logistic regression models (child, school, classroom) including significant exposure variables from first model, all personal factors and all environment factors. No results reported for rhinitis and formaldehyde because it wasn't significantly associated with rhinitis in the first model.		
Palczynski et al.	Random sample of 120 households with children ages	24-hr household sample, area	Allergy: 5 allergen skin prick test (dust, dust mites,	Environmental tobacco smoke	Contingency table analysis, prevalence (n, %)	278 adults, 186 children	**Allergy (skin prick tests), children**

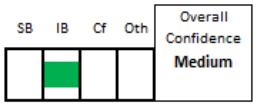
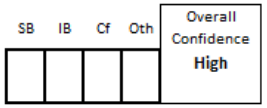
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
(1999) (Poland) Residences: adults, children (prevalence survey)	5–15 yrs, built 10 yrs before study. Participation rate not reported (i.e., were more than 120 households originally recruited?)	not specified; up to 0.067 mg/m^3 (most <0.050). Calibration 0.005 to 0.100 mg/m^3	feathers, grasses); serum IgE positive if ≥ 0.35 kU/l RAST. Asthma: Bronchial asthma diagnosis based on American Thoracic Society criteria (Ferris, 1978) (additional details not reported). Diagnosis interpreted to be for current status. Exposure measurement blinded to outcome classification		by age (adult; children) exposure group, and environmental tobacco smoke exposure. Highest exposure group very sparse.		 Uncertainty about time window of exposure measurement with respect to skin prick test results. **Allergy (skin prick tests) in adults**  Uncertainty about time window of exposure measurement with respect to skin prick test results (greater uncertainty in adults than in children) **Asthma, children and adults**  Uncertainty regarding asthma definition All outcomes Not informative above 0.050 mg/m^3 because of sample size (≤5).

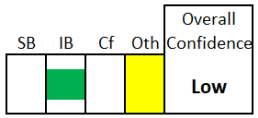
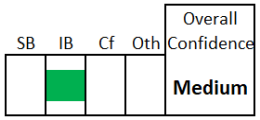
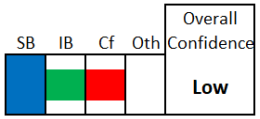
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Raaschou-Nielsen et al. (2010) (Denmark) Infants (birth cohort) 1998–2003	Copenhagen Prospective Study on Asthma in Childhood. 378 out of 411 (92%) participants at 18-mo follow-up; 343 with formaldehyde data.	Three 10-wk bedroom sampling periods from birth to 18 mos (aimed for 6, 12, and 18 mos). Median 0.018 mg/m^3, 95th percentile 0.037 mg/m^3. Within individual variance 69% of total variance	Daily diary kept by parents on respiratory symptoms. Training and definitions provided. Wheezing = any symptom severely affecting the child's breathing, such as noisy breathing (wheeze or whistling sounds), breathlessness, shortness of breath, or persistent, troublesome cough). Reviewed by study personnel every 6th month and after a 3-day period of respiratory symptoms. Outcome defined as "ever had at least one symptom day"; sensitivity analysis defined outcome as three or more consecutive days with wheezing symptoms.	Adjusted for sex, area of residence, education of mother, baseline lung function	Logistic regression of "ever had at least one symptom day" (88% = yes) and linear regression of number of symptom days (excluded 78 with 0 d). Analyzed by quintile of exposure (reference = <0.012 mg/m^3)	343	**Lower respiratory tract symptoms in infants and toddlers**  Analysis does not take into account important features of the data (e.g., temporal variations in symptoms and large within individual variability formaldehyde); could have masked an association
Roda et al. (2011) (France) Residences: infants (birth cohort) 2003–2006	Infants (singletons, >2,500 g) from 5 maternity hospitals in Paris. N = 3,840 out of 4,177 (92%) initially enrolled completed 1 or more questionnaires;	Questionnaire on home characteristics at baseline and updated at 3, 6, 9, and 12 months. N = 196 randomly selected for predictive modeling	Parent questionnaire at 1, 3, 6, 9, and 12 months: •Upper respiratory infections •Lower respiratory infections •Eczema	Examined sex, older sibling, parental asthma, history, socioeconomic status (4 levels, based on parents' occupation), prenatal and postnaltal tobacco smoke	Exposure prediction model for high versus low (based on median): sensitivity 72.4% specificity 73.6%. Exposure prediction model by tertile:	2,940	**Lower respiratory tract symptoms in infants and toddlers**  Did not test predictive model on separate sample (may overestimate sensitivity and specificity)

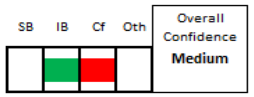
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	2,940 had baseline and 12 month questionnaire (70% of initial enrollees; 76% of those with 1 or more questionnaire)	analysis. Based on 4 1-wk measures at 1, 3, 6, and 9 months. LOD 0.008 mg/m^3. Median 0.020 mg/m^3; IQR 0.014, 0.027 mg/m^3. Predictors included measures of continuous formaldehyde exposure, intermittent exposure, home characteristics, and air flow	•wheezing episodes (frequency) •At 12 mos, also includes shortness of breath, dyspnea, dry cough at night without cold Used to define lower respiratory infections with and without wheeze	exposure, dampness, breast feeding <3 mos, day care, pets in home	sensitivity 57.4% specificity 82.1%. Outcome examined as LRI versus no LRI, and as 3-level variable in multinominal logistic regression (LRI-with wheeze; LRI-no wheeze, no LRI)		
Rumchev et al. (2002) (Australia) Residences: children (case-control) **Related reference:** Rumchev et al. (2004)	Limited to ages 6-36 mos; recruitment process not described for cases or controls; cases from emergency room and controls (age matched) from area health department, representing the catchment area of the hospital	8-hr samples, bedroom and living room, two seasons. Mean 0.030 and 0.28 and maximum 0.224 and 0.190 mg/m^3, respectively, in bedroom and living room.	Emergency room discharge diagnosis of asthma, ages 6–36 mos.	Adjusted or considered age, allergies, family history of asthma, dust mites, relative humidity, temperature, atopy, environmental tobacco smoke, pets, air conditioning, use of gas appliances	Generalized estimating equation modeling for repeated measures	88 cases, 104 controls	**Lower respiratory tract symptoms in infants and toddlers**  Recruitment process not described; uncertainty as to what is included within this case definition and length of time between emergency room visit and subsequent exposure measure.

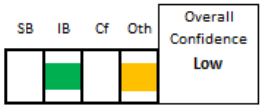
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Smedje and Norback (2001) (Sweden) Schools: children (nested case-control design) 1993–1997 **Related reference:** Smedje et al. (1997); however, this baseline study of prevalence of current asthma used measures taken in 1993, which ranged from <0.005 to 0.010 mg/m^3, with >50% less than LOD. Thus, this analysis did not meet EPA's	Nested case-control in school-based cohort study, 1st, 4th, and 7th grades at baseline (1993); follow-up in 1997. Excluded if history of allergy at baseline. 78% participation in follow-up. Schools randomly selected in Uppsala, Sweden; 2–5 classrooms selected from schools for exposure measures. Participants compared to nonparticipants on baseline characteristics.	4-hr (school day) samples, 2–5 rooms per school (chose frequently used rooms), 1993 and 1995; <0.005 to 0.042 mg/m^3. Mean 0.008, geometric mean 0.004 mg/m^3	Allergy: Parent report of incident allergy to hay fever/pollen or pet dander. Asthma: Parent-report of incident physician diagnosis (validation study: specificity >99%, sensitivity 73% compared with physician's assessment). Exposure measurement blinded to outcome classification	Adjusted for age, sex, history of atopy (eczema) at baseline, changes in smoking habits. Collinearity among measures (including VOC, mold) assessed; did not attempt adjustment for multiple exposures but pattern of results differed among the exposures examined.	Logistic regression, OR (95% CI) per 0.010 mg/m^3 increase [high proportion below detection limit of 0.005 mg/m^3, 54% of 1993 samples and 24% of 1997]. Results similar when students who were no longer in the school excluded (about 2/3 left the school at mean of 1.5 yrs before follow-up)	88 incident pollen allergy; 50 incident pet allergy cases; 56 incident asthma cases out of 1,258 at baseline.	**Allergy (incidence of allergies) and asthma (incidence)**  Exposure measures in only 2 of the 4 yrs; uncertainty about distribution; relatively high percentage <LOD. Confounding by other exposures not fully addressed but pattern of results differed among the exposures examined. Alternative evaluation: Medium confidence (based on strengths of prospective study of incidence) (*Information on percent below detection limit and individual student exposures provided in email from Dr. Greta Smedje, March 22, 2012)*

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
inclusion criteria.							
Tavernier et al. (2006) (United Kingdom) Residences: children (case-control) **Related reference:** Gee et al. (2005)	Cases from two primary care practices, age- and sex-matched controls from same practices. Ages 4–17 yrs. Participation rate 50%. 95 additional cases excluded because no matching control identified. [Note: Gee et al. (2005) described the age range as 4–16 yrs]	7-d sample in living room and bedroom. Did not report any information on exposure distribution. [Note: Gee et al. (2005) described this as a 5 d sample; median values 0.037 and 0.049 mg/m^3 in living room and bedroom, respectively]	Positive responses to three questions on screening questionnaire: (1) wheezed in the last 12 mos; (2) woken at night by cough in the absence of a cold or respiratory infection in the last 12 mos; (3) received more than three courses of antibiotics for respiratory symptoms (both upper and lower respiratory tract) in the last 12 mos; (4) history of hay fever or eczema; (5) family history of asthma in first degree relatives. In validation study, positive predictive value 84% for meriting trial for asthma medication. Exposure measurement blinded to outcome classification. [Note: Gee et al. (2005) described the positive predictive value from the validation study as 79%]	Adjusted for measured exposures (e.g., endotoxin, Der p 1, particulate matter, NO_2, and other risk factors.	Logistic regression, OR (95% CI) by tertile (but exposure levels by tertile not reported)	105 cases, 95 controls	**Asthma**  Uncertainty regarding selection process and loss of almost half of the cases. Outcome classification includes questions that are not specific to asthma. Uncertainty as to exposure range, particularly upper tertile (no response from email to corresponding author).

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Venn et al. (2003) (United Kingdom) Residences: children (case-control and symptom control among cases) October–May 1998 **Related reference:** Venn et al. (2000)	Participants in air pollution study 1993–1995, 85% response rate; 835 potential cases (positive wheeze question) and 860 potential controls recontacted in 1998; 54% responded. From this, 243 eligible cases and 383 eligible controls identified. Participation rate 79% cases, 59% controls.	3-d sample in bedroom in 1998 concurrent with data collection on outcomes; median 22 µg/m^3; 75th percentile 32 µg/m^3	Asthma: Parent report of persistent wheeze (1995–1996 and 1998); validation by medical record review of prescription for asthma medication. Symptom frequency: One month daily diaries recording symptoms, including daytime and nighttime wheezing, chest tightness, breathlessness, and cough, each measured on 0 to 5 scale. Exposure measurement blinded to outcome classification	Adjusted for age, sex, Carstairs deprivation index (based on postal code). Also examined and addressed other variables, including NO_2, moisture, mold, season	Logistic regression, OR (95% CI) by quartile. Examined effect modification of symptom frequency by atopy	190 cases, 214 controls	**Asthma**  Uncertainty about time window of exposure measure **Asthma control** 
Yeatts et al. (2012) (United Arab Emirates) Residences (survey) October 2009 to May 2010	Nationally representative sample of households, stratified by geographic area and population density. 628 households, household participation rate 75%. Age-stratified sample selected from households.	7-d sample in living room. 71% <LOQ (0.0074 mg/m^3); 95th percentile 0.059 mg/m^3; 99th percentile 0.114 mg/m^3 (converted from ppm) .	Symptom questionnaire (last 4 wks), drawn from standard questionnaires. Mothers responded for children. Exposure measurement blinded to outcome classification	Moderate correlation between formaldehyde and sulfur dioxide (r = 0.63); formaldehyde strongly associated with frequency of incense use. Adjusted for sex, urban/rural area, age group, household tobacco smoke exposure.	Logistic regression, above versus below detection limit, OR (95% CI)	1007 adults, 330 ages 11–18 years, 253 ages 6–10 years	**Asthma -children and adults (combined)**  Difficult to disentangle possible effects of sulfur dioxide from those of formaldehyde (similar effect sizes; moderate-strong correlation; could result in inflated effect estimate. Does not separate analysis of children and adults; only 29% above LOD—

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
							analyzed as above versus below LOD
Yon et al. (2019) (Seongnam City, Korea) Cross-sectional	5th and 6th grade students were recruited from 22 randomly selected classrooms at 11 elementary schools (n = 620), aged 10–12 yr. A total of 427 children participated (68.9%).	Formaldehyde sampling in each classroom using monitors with pumps during the 1st and 2nd half of the school year. Mean 27.17 ± 7.72 µg/m^3; as high as 60 µg/m^3 in some classrooms. Duration and sampling methods were not described.	Current asthma or rhinitis definition: presence of characteristic symptoms and /or signs during the previous 12 mos using ISAAC questionnaire, Self report. Rhinitis severity categorized using Allergic Rhinitis and Its Impact on Asthma guidelines. Current asthma n = 10 Rhinitis n = 246	Models for asthma or rhinitis adjusted for age and sex apriori. Also adjusted for variables based on statistical significance in model ($p < 0.10$). Covariates were BMI z-score, height, prematurity or low birth weight, home renovation, environmental tobacco smoke, keeping a pet at home, and physician-diagnosed atopic dermatitis, allergic rhinitis, and parental asthma	Analysis used generalized linear mixed models with robust variance estimates and post hoc Bonferroni correction. Accounted for classroom (random effect)	N = 427	**Current asthma**  **Low** Few children with asthma contributed to analyses **Rhinitis in last 12 months and rhinitis severity**  **Medium** Reporting deficiencies raise concern for bias in exposure measurement, sampling duration and methods not described.
Yu et al. (2017) (Hong Kong) Birth cohort November 2009 to April 2011	702 of 2,423 (29%) eligible infants aged ≤ 4 mos attending 29 maternal and child health centers between November 2009 to April 2011, stratified by family	Air sampling (NO_2, formaldehyde) using standardized diffusion samplers at 6 mos of age in bedroom.	Baseline information obtained using validated ISAAC questionnaire completed by parents prior to age 4 mos. Weekly respiratory health diary and monthly health telephone survey	Potential confounders selected from baseline characteristics associated with formaldehyde concentrations using log-rank test, $p < 0.25$.	Cox regression in entire sample; formaldehyde modeling as continuous variable; effect modification by family history was analyzed.	N = 535	**New onset wheezing** Infants  **Low** No details provided for exposure measurements;

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	history of asthma, family history of allergy and no family history. Enrollment numbers based on power calculations. A total of 535 with complete air sampling for NO_2 and HCHO. No comparisons of participants with nonparticipants.	Mean (SD) concentrations NO_2 42.4 (30.97) µg/m^3; HCHO 51.09 (74.94) µg/m^3; no details regarding sampling methods or duration.	blinded to exposure status until 18 mos of age. New onset wheeze measured from 6 to 18 mos of age. 120 (11%) infants had new onset wheeze at an average of 11.4 mos.	Stepwise adjustment, final models adjusted for NO_2, neonatal respiratory illness, having a sibling, family history allergy or asthma, living area, pets, or cooking fuel.			concern for selection bias. Participation rate was very low (29% of eligible agreed) and of those selected there was notable data loss, data was complete for 76%. No comparisons of participants and nonparticipants. No control for ETS
Zhai et al. (2013) (China) Residences (survey) January 2008 to December 2009	Provided criteria for selection of homes in defined area; evaluated 186 homes in Shenyang, China; homes were decorated in last 4 yrs and occupied within the last 3 yrs. Participation rate of households not reported (i.e., were more than 186 households originally recruited?) Participants within houses were randomly selected	Cited Code for indoor environmental pollution control of civil building engineering (GB50325-2001); samples in 3 rooms per house (bedroom, living room, kitchen); sampling time not specified (no response from email to corresponding author); N=558 samples in 186 homes.	Asthma: based on American Thoracic Society (Ferris, 1978) questionnaire	Univariate analysis; confounding unlikely explanation of the results in children	Univariate results for asthma outcome [multivariate modeling of "respiratory symptoms"; not clear what is included in this category)	186 homes 186 adults, 82 children	**Asthma Children**  Uncertainty regarding exposure measurement period. Although potential confounders were not considered in asthma only analysis, the magnitude of the results is unlikely to be explained by confounders. **Adults**

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		Exposure groups "polluted" homes: >0.08 mg/m^3, mean 0.09–0.13 mg/m^3 in three rooms; "nonpolluted" ≤0.08 mg/m^3, mean 0.04–0.047 mg/m^3. 64% of the 186 houses, and 24% of the 82 houses with children were >0.08 mg/m^3 ("polluted")					 See notes above, for children. In addition, for adults. small number of positive responses.

1

Evaluation of Controlled Exposure Studies

The evaluation of controlled exposure studies examined four primary elements: the type of exposure (paraformaldehyde preferred over formalin or undefined test articles), use of randomization procedures to allocate exposure, blinding of the participant and of the assessor to exposure, and the details regarding the analysis and presentation of results. The subsequent table in this section provides the more detailed documentation of the evaluation of controlled human exposure studies (see Table A-52); studies are arranged alphabetically within this table.

Table A-52. Evaluation of controlled acute exposure studies among people with asthma

Reference	Exposure assessment	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure)	Consideration of likely confounding	Results presentation	Size	Confidence
Casset et al. (2006)	Formalin, 30 min, 0.032 (background) and 0.092 mg/m^3, achieved concentrations analyzed. Includes allergy challenge. Nose clipped during exposure (mouth breathing)	Spirometry; FEV_1, FEF_{25-75}, PEF (protocol not mentioned) and bronchial challenge-airway reactivity test (PD_{20} FEV_1 Der p1) (standard protocol) Testing pre- and every hour up to 6 hrs postexposure.	Mild asthma, ages 19–35 yrs, no respiratory infections for 2 wks; not in relevant allergy season or living with a pet if allergic. Random assignment to order of exposure (3 wks between experiments); double blinded	Within-person	Individual data values and *t*-tests	19	Overall Confidence **High** Randomized, double blinded, detailed data presentation; applies to mouth breathing
Ezratty et al. (2007)	Formalin, 60 min, 0 and 0.500 mg/m^3, achieved concentrations analyzed. Includes allergy challenge	Spirometry; FVC, FEV_1 (ECRHS protocol), and bronchial challenge-airway reactivity test (PD_{15} FEV_1 grass) (standard protocol) Testing pre- and every hour up to 6 hrs postexposure.	Intermittent asthma (dyspnea < twice per week and night symptoms < twice per month with PEF > 80%), ages 18–45 yrs; not in allergy season. Random assignment to order of exposure (2 wks between experiments); double blinded.	Within-person	Individual data values and Wilcoxon sign rank test	12	Overall Confidence **High** Randomized, double blinded, detailed data presentation

Reference	Exposure assessment	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure)	Consideration of likely confounding	Results presentation	Size	Confidence
Green et al. (1987)	Paraformaldehyde, 60 min, clean air and 3 ppm, achieved concentrations analyzed.	Spirometry; FVC, FEV_1, SGaw (ATS protocol), testing pre- and during exposure period, ≈15 min intervals.	Asthma (clinical history), no respiratory infection for 2 wks, age 19–35 yrs. Random assignment to order of exposure; two 15-min exercise segments in 60-min exposure period; single blinded +	Within person	Group means and SE	16	Overall Confidence **Medium** Randomized, single blinded
Harving et al. (1990) **Related Reference:** Harving et al. (1986)	Formalin, 90 min, filtered air (8), 0.120 and 0.850 mg/m^3, achieved concentrations analyzed.	Spirometry; FEV_1, R_{aw}, SGaw (protocol not mentioned), testing pre- and near end of exposure period. Bronchial challenge-airway reactivity test, immediately after exposure PEF by home peak flowmeter every 2 hrs after exposure and next morning	Asthma (substantial bronchial hyperreactivity to histamine), age 15–36 yrs. Random assignment to exposure order (one per week); double blinded	Within-person	Group means and SD	15	Overall Confidence **High** Randomized, double blinded, detailed analysis

Reference	Exposure assessment	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure)	Consideration of likely confounding	Results presentation	Size	Confidence
Krakowiak et al. (1998)	Formalin, 2 hrs, 0.5 mg/m^3, achieved concentrations analyzed.	Spirometry FEV_1 (testing 2 hrs pre- and immediately after, 5 hr, and 24 hr) PEF (testing at beginning of exposure, every hour for 12 hrs, 24 hrs after)	Formaldehyde-exposed workers with asthma. Order not randomized (1 wk between experiments); single blinded	Within person	Group means (bar graph)	10	Overall Confidence Low Not randomized, single blinding, SE or SD not reported
Sauder et al. (1987)	Paraformaldehyde, 3 hrs, clean air and 3 ppm, achieved concentrations analyzed.	Spirometry; FVC, FEV_1, SGaw (ATS protocol), testing at 0, 15, 30, 60, 120, 180 min during exposure.	Asthma (clinical history), no respiratory infection for 6 wks, age 26–40 yrs. Order not randomized; clean air followed by formaldehyde (one week apart); blinding not specified	Within person	Grouped means and paired *t*-tests for most measures, individual FEV_1 data	9	Overall Confidence Low Not randomized, blinding not specified
Sheppard et al. (1984)	Paraformaldehyde, 10 min, 0, 1, and 3 ppm, achieved concentrations analyzed.	Spirometry; SGaw, testing before and 2 min after exposure.	Asthma (clinical history), age 18–37 yrs. Randomization of order not reported; two protocols (at rest and during exercise) ≥1 d apart; blinding not specified	Within person	Grouped means and SD and paired *t*-tests	7	Overall Confidence Low Randomization and blinding not specified

Reference	Exposure assessment	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure)	Consideration of likely confounding	Results presentation	Size	Confidence
Witek et al. (1987); Witek et al. (1986)	Paraformaldehyde (with 2-propanol?), 40 min, 0 and 2 ppm	Spirometry; FVC, FEV_1, R_{aw}, testing during and at 10 and 30 min postexposure; PEFR assessed from 1 to 24 hrs post exposure.	Mild asthma (ATS definition), age 18–35 yrs. Random assignment to order of exposure; two protocols (at rest and during exercise); double blinded	Within person	Individual data values and paired *t*-test	15	Overall Confidence High Randomized, double blinded; nonparametric analysis could be preferred but individual data provided

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Experimental Animal Studies

The experimental animal studies identified as a result of the literature search specific to this section are evaluated as mechanistic information in Appendix A.5.6.

A.5.5. Respiratory Tract Pathology

Literature Search

Studies in Humans

A systematic evaluation of the literature database on studies examining the potential for respiratory tract pathology in humans in relation to formaldehyde exposure was initially conducted in September 2012, with regular updates to September 2016 as described elsewhere (see Appendix A.5.1 and a separate Systematic Evidence Map that updates the literature from 2017–2021 using parallel approaches; see Appendix F). The search strings used in specific databases are shown in Table A-53. Additional search strategies included:

- Review of reference lists in the articles identified through the full screening process and
- Review of reference lists in the 2010 draft Toxicological Review for Formaldehyde (U.S. EPA, 2010).

This review focused on histopathological endpoints and signs of pathology in nasal tissues. Inclusion and exclusion criteria used in the screening step are described in Table A-54. The search and screening strategy, including exclusion categories applied and the number of articles excluded within each exclusion category, is summarized in Figure A-28. Based on this process, as of the last literature search update, 12 studies were identified and evaluated for consideration in the Toxicological Review.

Table A-53. Summary of search terms for respiratory tract pathology in humans

1. Database, 2. Initial Search Date	3. Terms
PubMed 12/18/2012 No date limitation	(Formaldehyde[majr] OR paraformaldehyde[majr] OR formalin[majr]) AND (Hyperplasia OR metaplasia OR nasal mucosa OR occupational diseases OR respiratory tract diseases OR rhinitis OR mucociliary) AND (epidemiology OR epidemiological OR epidemiologic OR cohort OR retrospective studies OR retrospective OR prospective studies OR prospective OR cross-sectional OR case-control OR cross-sectional study OR prevalence study OR occupational)
Web of Science 12/19/2012 No date limitation	TS=(Formaldehyde OR paraformaldehyde OR formalin) AND TS=(Hyperplasia OR metaplasia OR nasal mucosa OR occupational diseases OR respiratory tract diseases OR rhinitis OR mucociliary) and TS=(epidemiology OR epidemiological OR epidemiologic OR cohort OR retrospective studies OR retrospective OR prospective

1. Database, 2. Initial Search Date	3. Terms
	studies OR prospective OR cross-sectional OR case-control OR cross-sectional study OR prevalence study OR occupational)
Toxline 05/03/2013 No date limitation	(Formaldehyde OR Paraformaldehyde OR Formalin) AND (Hyperplasia OR metaplasia OR nasal mucosa OR occupational diseases OR respiratory tract diseases OR rhinitis OR mucociliary) AND (epidemiology OR epidemiological OR epidemiologic OR ohort OR retrospective studies OR retrospective OR prospective studies OR prospective OR cross-sectional OR case-control OR cross-sectional study OR prevalence study OR occupational)

Table A-54. Inclusion and exclusion criteria for studies of repiratory pathology in humans

	Included	Excluded
Population	• Humans	• Animals
Exposure	• Indoor exposure via inhalation to formaldehyde • Measurements of formaldehyde concentration in air	• Not about formaldehyde • Not inhalation (e.g., dermal exposure)
Comparison	• Evaluated outcome associations with formaldehyde exposure	• Case reports • Surveillance analysis/Illness investigation (no comparison)
Outcome	• Histopathology and signs of pathology in nasal tissues	• Other health endpoints • Nasal symptoms (e.g., rhinitis, mucous flow rate) • Not a health study • Exposure studies/no outcomes evaluated
Other		• Reviews and reports (not primary research), letters, meeting abstract, no abstract, methodology paper, nonessential article in a foreign language (e.g., after review of title and abstract, if available, or consultation with native speaker)

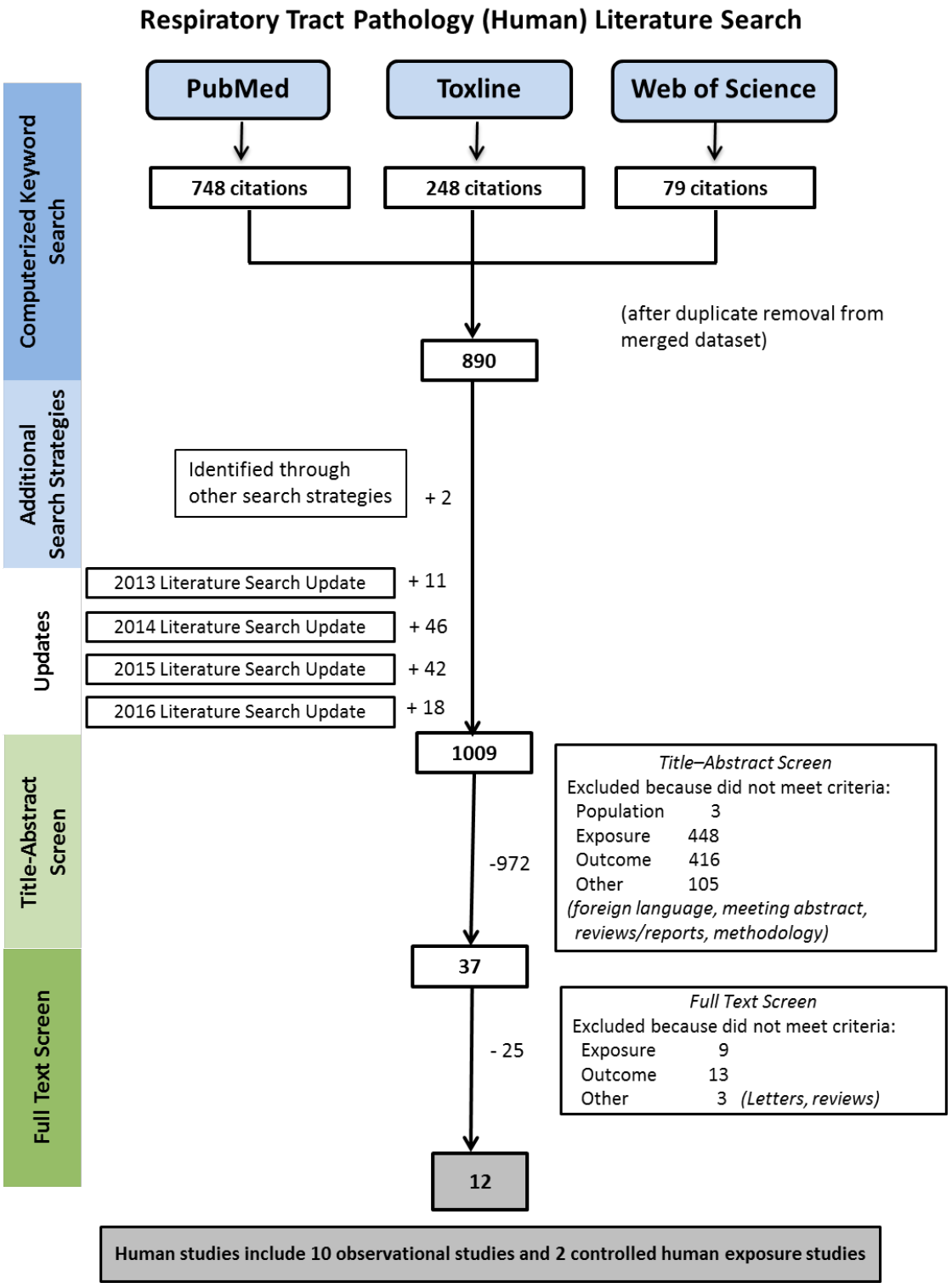


Figure A-28. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and respiratory tract pathology in humans (reflects studies identified in searches conducted through September 2016).

Studies in Animals

A systematic evaluation of the literature database on studies examining the potential for respiratory tract pathology in animals in relation to formaldehyde exposure was initially conducted in September 2012, with regular updates as described elsewhere. The search strings used in specific databases are shown in Table A-55. Additional search strategies included:

- Review of reference lists in the the articles identified through the full screening process,
- Review of reference lists in the 2010 draft Toxicological Review for Formaldehyde (U.S. EPA, 2010), and
- Review of references in 6 review articles relating to formaldehyde and respiratory pathology in animals, published in English, identified in the initial database search.

Inclusion and exclusion criteria used in the screening step are described in Table A-56. After manual review and removal of duplication citations, the 1,631 articles were initially screened within an EndNote library; title was considered first, and then abstract in this process. Full text review was conducted on 105 identified articles. The search and screening strategy, including exclusion categories applied and the number of articles excluded within each exclusion category, is summarized in Figure A-29. Based on this process, 41 studies were identified and evaluated for consideration in the respiratory tract pathology section of the Toxicological Review. An additional 35 studies related to MOA for pathology were considered in the overarching mechanistic evaluation (see Appendix A.5.6).

Table A-55. Summary of search terms for respiratory tract pathology in animals

Database, initial search date	Terms
PubMed 10/18/2012 Search up through 9/30/2012	Formaldehyde* AND (animals OR dog OR dogs OR canine OR canines OR beagle OR beagles OR "guinea pig" OR "guinea pigs" OR Cavia OR hamster OR hamsters OR Cricetinae OR Mesocricetus OR mice OR mouse OR Mus OR monkey OR monkeys OR Macaca OR primate OR primates OR rabbit OR rabbits OR hare OR hares OR rat OR rats OR Rattus OR Rana or rodent OR rodents OR Rodentia) AND (alveol* OR bronchial OR bronchi OR buccal OR laryngeal OR larynx OR lung OR mouth OR nasal OR nasopharyngeal OR nasopharynx OR nose OR pharyngeal OR pharynx OR pulmonary OR respiratory OR sinonasal OR sinus OR trachea*) AND (edema OR oedema OR cancer OR carcinogens OR carcinogenesis OR carcinogenicity OR carcinoma OR "cell proliferation" OR cilia OR dysplas* OR epithelial OR epithelium OR goblet OR histopath* OR hyperplas* OR hypertrophy* OR metaplas* OR mucociliary OR mucos* OR mucous OR mucus OR necrosis OR neopla* OR olfactory OR patholog* OR rhinitis OR squamous OR transitional OR tumor OR tumour OR turbinate OR ulceration) NOT human
Web of Science 10/18/2012 Search up through 9/30/2012	Topic=Formaldehyde* AND (animals OR dog OR dogs OR canine OR canines OR beagle OR beagles OR "guinea pig" OR "guinea pigs" OR Cavia OR hamster OR hamsters OR Cricetinae OR Mesocricetus OR mice OR mouse OR Mus OR monkey OR monkeys OR Macaca OR primate OR primates OR rabbit OR rabbits OR hare OR hares OR rat OR rats OR Rattus OR

Database, initial search date	Terms
	Rana or rodent OR rodents OR Rodentia) AND (alveol* OR bronchial OR bronchi OR buccal OR laryngeal OR larynx OR lung OR mouth OR nasal OR nasopharyngeal OR nasopharynx OR nose OR pharyngeal OR pharynx OR pulmonary OR respiratory OR sinonasal OR sinus OR trachea*) AND (edema OR oedema OR cancer OR carcinogens OR carcinogenesis OR carcinogenicity OR carcinoma OR "cell proliferation" OR cilia OR dysplas* OR epithelial OR epithelium OR goblet OR histopath* OR hyperplas* OR hypertrophy* OR metaplas* OR mucociliary OR mucos* OR mucous OR mucus OR necrosis OR neopla* OR olfactory OR patholog* OR rhinitis OR squamous OR transitional OR tumor OR tumour OR turbinate OR ulceration) NOT human
Toxline 10/21/2012 Search up through 9/30/2012	formaldehyde AND (animal OR "nasal cavity" OR nose OR "respiratory tract" OR "cell proliferation" OR mucociliary OR histopathology OR pathology OR cancer OR tumor) NOT (human OR humans OR epidemiology OR epidemiological OR occupation* OR work* OR antinocicepti* OR nocicepti* OR pain OR sensory OR "formalin test" OR bacteria OR bacterial) (including synonyms and CAS numbers, but excluding PubMed records)

Table A-56. Inclusion and exclusion criteria for studies of repiratory pathology in animals

	Included	Excluded
Population	Animals	Irrelevant species/ matrix, or human studies
Exposure	Inhalation exposure, formaldehyde or test article generating formaldehyde	Not formaldehyde (or formaldehyde exposure not quantified: full text screening only) Dermal or oral exposure or other noninhalation exposure Endogenous properties
Comparison		
Outcome	Respiratory tract pathology MOA for pathology (note: these are evaluated and discussed in the overarching MOA section; see A.1.6)	Assessment of formaldehyde exposure Chemical properties Formaldehyde use in methodology or treatement Not related to respiratory tract pathology
Other		Reviews and reports (not primary research), letters, meeting abstract, policy/ current practice paper, duplicate, nonessential article in a foreign language

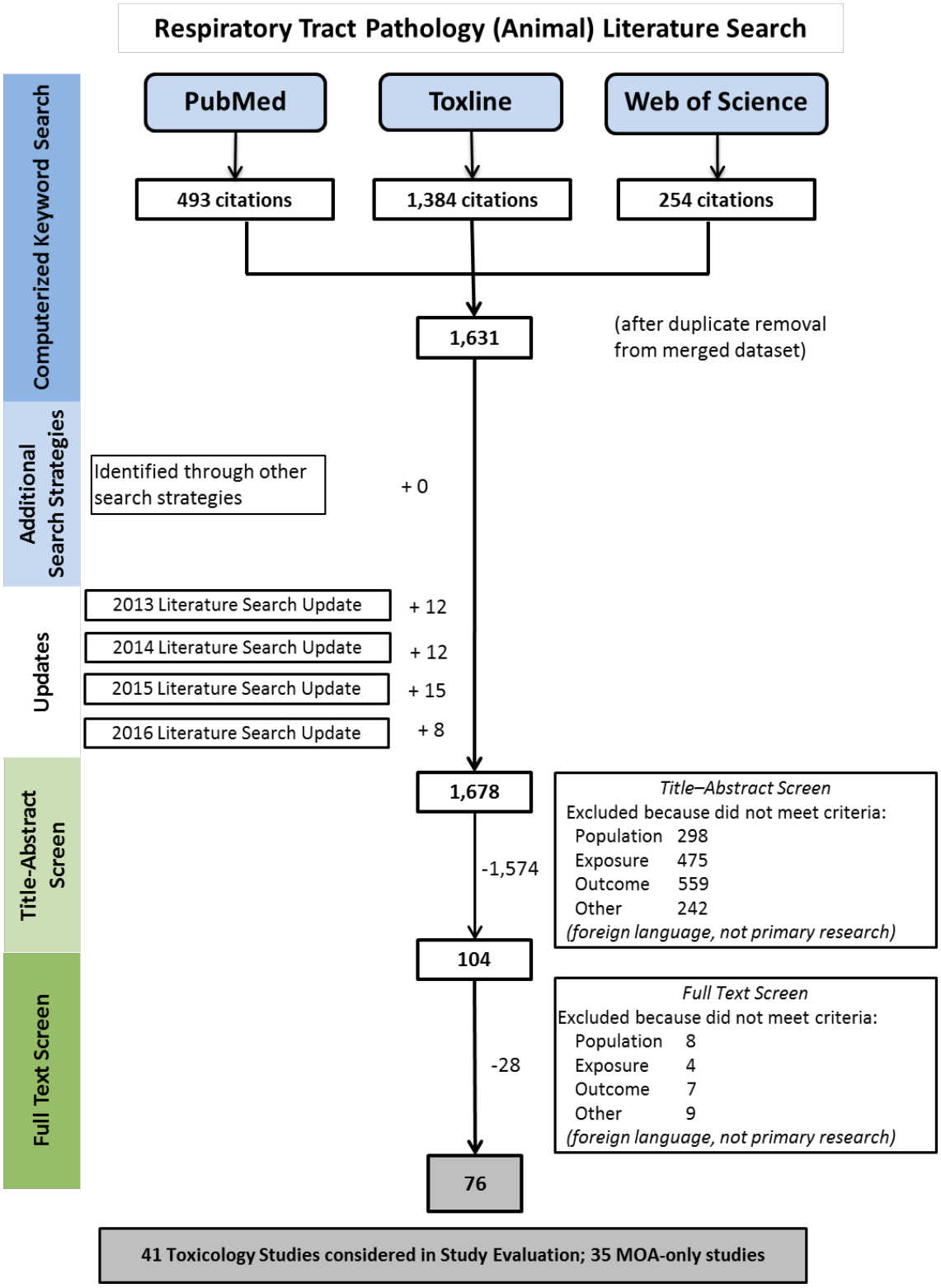


Figure A-29. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and respiratory tract pathology in animals (reflects studies identified in searches conducted through September 2016).

Study Evaluations

Studies in Humans

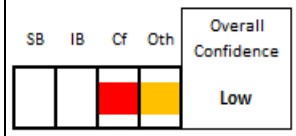
Each study was evaluated for precision and accuracy of exposure assessment, measurement of outcome, participant selection and comparability, possibility of confounding, analysis and completeness of results, and study size (see Table A-57). The accompanying tables in this section document the evaluation. Studies are arranged alphabetically within each table.

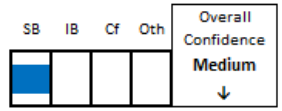
For studies that evaluated histopathological lesions in nasal biopsies, EPA looked for either a detailed explanation of how tissues were evaluated and scored, or a citation for a standard method. Cross-sectional studies among occupational cohorts likely were influenced by the selection of the workforce toward individuals less responsive to the irritant properties of formaldehyde, with a reduction in sensitivity. These studies were downgraded because of this limitation. Treatment of potential confounding by studies also was evaluated. EPA considered age, gender and smoking to be important confounders to evaluate for effects on pathological endpoints. EPA also looked for consideration of confounding by other co-exposures in the workplace depending on the occupational setting.

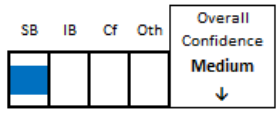
Table A-57. Criteria for categorizing study confidence in epidemiology studies of respiratory pathology

Confidence	Exposure	Study design and analysis
High	**Work settings:** Ability to differentiate between exposed and unexposed, or between low and high exposure.	Selection of workers at beginning of exposures (no lead time bias). Instrument for data collection described or reference provided and outcome measurement conducted without knowledge of exposure status. Analytic approach evaluating dose-response relationship using analytic procedures that are suitable for the type of data, and quantitative results provided. Confounding considered and addressed in design or analysis; large sample size (number of cases).
Medium	**Work settings**: Referent group may be exposed to formaldehyde or to other exposures affecting respiratory conditions (potentially leading to attenuated risk estimates).	Lead time bias may be a limitation for occupational studies. Instrument for data collection described or reference provided and outcome measurement conducted without knowledge of exposure status. Analytic approach more limited; confounding considered and addressed in design or analysis but some questions regarding degree of correlation between formaldehyde and other exposures may remain. Sample size may be a limitation.
Low	Work settings: Short sampling duration (<1 work shift) without description of protocol. Missing values or values <LOD for large proportion of subjects.	Lead time bias may be a limitation for occupational studies. High likelihood of confounding that prevents differentiation of effect of formaldehyde from effect of other exposure(s), limited data analysis (or analysis that is not appropriate for the data) or small sample size (number of cases).
Not informative	Exposure range does not allow meaningful analysis of risks above 0.010 mg/m^3; no information provided.	Description of methods too sparse to allow evaluation.

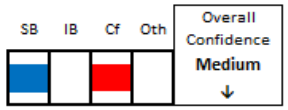
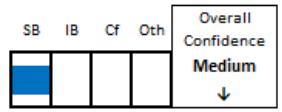
Table A-58. Respiratory pathology

Reference	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Size/ estimated power	Comments
School Settings							
Norback et al. (2000) (cross-sectional study)	Exposure measurements in 2 randomly selected classrooms at each school on 2 occasions; Measurements of respirable dust, CO2, temperature, humidity, formaldehyde (4-hour sample), airborne microorganisms, viable molds and bacteria, NO_2 (only in 1993); all staff assigned school mean concentration. Formaldehyde concentration: mean 0.0095 mg/m^3; min-max of means, 0.003−0.016 mg/m^3; provided citation for analysis; LOD 0.005 mg/m^3 (Smedje et al., 1997)	Interview for symptoms, nasal lavage and acoustic rhinometry; use of both subjective and objective measures enabled evaluation of information bias	Primary school personnel at 12 of 18 randomly selected schools (out of 62) and with restriction to schools with classes 1−6 and no changes in ventilation or redecoration during study period (March 1993−March 1995). 234 current employees (84%) working 20 hr/wk or more. Excluded those on sick leave or otherwise off duty. High participation reduced likelihood of selection bias.	Multiple linear regression models adjusted for age, sex, smoking, atopy, and mean classroom temperature; Co-exposure: Nasal patency measures were inversely associated with dust, NO_2, and Aspergillus. Elevations in nasal lavage biomarkers associated with NO_2, Aspergillus, and yeast; correlation between indoor levels of pollutants or microbials not reported; correlated with ventilation? No	Multiple linear regression models; reported regression coefficients and whether statistically significant (p <0.05); uncertainties in analysis: use of school-based mean concentration as unit of analysis	N = 234 individuals, but unit of analysis was school means, N = 12	 Unknown correlation between co-exposures (dust, NO_2, and Aspergillus) which also were inversely associated with nasal patency and biomarkers, potential confounding; some schools with mean < LOD; less robust analytic approach given unit of analysis

Reference	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Size/ estimated power	Comments
				indoor sources of combustion—NO_2 levels higher in schools near traffic			
Occupational Settings							
Ballarin et al. (1992) Prevalence study	Personal sampling; 8-hr TWA (NIOSH, 1977) Warehouse (N = 3), 0.39 ± 0.20 mg/m^3, range 0.21–0.6 mg/m^3 Shearing-press (N = 8), 0.1 ± 0.02 mg/m^3, range 0.08–0.14 mg/m^3 Sawmill (N = 1), 0.09 mg/m^3 Inspirable wood dust: 0.11–0.69 mg/m^3, 0.73 in sawmill	Cytopathology analysis of nasal respiratory mucosa cells by two trained readers blinded to exposure status; scoring and classification analogous to Torjussen et al. (1979) and Edling et al. (1988); most severe score present assigned.	Participant selection and recruitment not described. Nonsmokers in plywood factory (N = 15) compared to nonsmoking university or hospital clerks (N = 15) matched by age and sex. Excluded heavy drinkers. Use of referent group with different occupations results in less similar comparison groups	Addressed potential confounding by age and sex through matching and smoking and heavy alcohol use by exclusion.	Mean histological scores in exposed and referent compared using Mann-Whitney U test and frequency by classification using chi-square test	15 exposed/ unexposed pairs	 Inclusion only of current workers raises possibility of healthy worker survival effect due to irritation effects
Berke (1987) Cross-sectional study	Exposure measurements since the mid 1970s using personal monitoring (monitoring protocol not described).	Clinical exam and nasal cytology by pathologist blind to exposure or clinical status. System for classifying atypical	Participant selection and recruitment not described. 52 volunteers from three paper plants	Mean age in exposed higher than employee referent group, comparable to additional white-	Exposed (Groups 1 and 2) compared to referent (Groups 3 and 4); chi-square test with	42 exposed, 10 employee referents, 28 white-collar referents	 Methods were not well described. Comparisons of

Reference	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Size/ estimated power	Comments
	Group 1 ranging from 0.02−1.3 ppm. Group 2 plant 0.05−2.0 ppm	and typical metaplasia not defined.	(currently employed, participation 95% of available exposed) 42 exposed, 10 referent workers. 28 additional referent white-collar employees (36% atypical squamous metaplasia in this group)—not representative?	collar referent group. Smoking prevalence 60% in Groups 1, 2, and 3; 20% in white-collar referent. Statistical analysis excluding smokers	adjustment for age and smoking; analysis of combined groups not appropriate (exposures different and very different demographic characteristics)		dissimilar groups. Nonstandard outcome definition and analyses that cannot be interpreted. Inclusion of only current workers and long duration of employment (mean >15 years) raises possibility of healthy worker survival effect
Boysen et al. (1990) Cross-sectional, study	Formaldehyde monitoring conducted after 1980. Before 1980, exposure assigned by plant health officer with knowledge of the production process, recent measurements, and worker sensations. Range of formaldehyde 0.5 ppm to >2 ppm (0.62−2.5 mg/m^3); no measurements in referent; however,	Slides evaluated by two authors blinded to clinical or occupational status. Histology: Scoring and classification of histologic samples per variation of Torjussen et al. (1979) protocol. Rhinoscopy: Scoring according to Boysen et al. (1982, 10117953)	37/74 volunteers from a chemical company producing formaldehyde (50% of exposed workforce). Referents: 37 age matched subjects without overt nasal disease (office staff, hospital laboratory personnel, and EN&T outpatients). Use of referent group	Exposed and referent comparable for age, smoking, or previous nasal disease.	Comparison of histological results between exposed and referent groups using Wilcoxon rank sum test, evaluated associations with age, smoking, intensity and duration of exposure; comparison of rhinoscopical results using chi-square test	37 exposed, 37 referents	 Inclusion only of current workers and long duration of employment raises possibility of healthy worker survival effect due to irritation effects

Reference	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Size/ estimated power	Comments
	exposure contrast likely adequate.		with different occupations results in less similar comparison groups				

Reference	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Size/ estimated power	Comments
Edling et al. (1988, 1987a) Prevalence Study Related studies: Odkvist et al. (1985)	Past TWA formaldehyde measurements by plant industrial hygienists sporadically between 1975 and 1983. Levels of FA in air ranged from 0.1–1.1 mg/m^3, with peaks up to 5 mg/m^3. No measurements available before 1975, but estimated levels higher during the 1960s and early 1970s. No measurements in referent; however, exposure contrast likely adequate.	Rhinoscopy: Nasal mucosa histological grading by pathologist blinded to exposure using Torjussen et al. (1979) grading system	75 of 104 exposed male factory workers from 3 plants (72% of eligible). Referents: 25 men with similar age and no known industrial exposures to formaldehyde; source of referent group not described. Evaluated characteristics of nonparticipants at 1 plant, age and exposure time similar, % with symptoms higher in nonparticipants, % smokers lower	Exposed mean age: 38 yrs; 35% smokers. Referent mean age: 35 years, 48% smokers. Histological score was higher among exposed smokers compared to ex-smokers and nonsmokers; possible confounder	Exposed groups compared to referent group using Wilcoxon rank sum test, no adjustment for age or smoking	75 exposed, 25 referents	 Inclusion of only current workers and long duration of employment (mean 10.5 yrs) and high prevalence of symptoms raises possibility of healthy worker survival effect due to irritation effects
Holmstrom et al. (1989c); Holmström and Wilhelmsson (1988)	Personal sampling in breathing zone for 1–2 hours in 1985. Chemical Plant: 0.05–0.5 mg/m^3, mean 0.26 [SD 0.17 mg/m^3]. Furniture Factory: 0.2–0.3 mg/m^3, mean 0.25	Nasal symptoms questionnaire, nasal volume flow rate using rhinomanometry; mucociliary clearance using green dye to measure time for	Participant selection and recruitment protocol not reported; excluded subjects with upper airway infections; nasal specimens in 62 of	Formaldehyde exposed were slightly younger than formaldehyde-dust exposed or referent; smoking status higher in	Compared exposure groups using 2-tailed *t*-test for symptoms, nasal flow rate, and histology, and chi-square test	N = 62 of 70 Group 1, N = 89 of 100 Group 2, N = 32 of 36 Referent	 Inclusion of only current workers and long duration of employment raises possibility of healthy worker

Reference	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Size/ estimated power	Comments
Cross-sectional study	[SD 0.05 mg/m^3]. Referent 0.09 mg/m^3 formaldehyde. Total dust and respirable dust also measured.	spot to reach rhinopharynx. Histological changes in nasal mucosa graded by a pathologist blind to exposure according to Torjussen et al. (1979)	70 formaldehyde exposed, 89 of 100 formaldehyde/ wood dust exposed, and 32 of 36 referents. Apparent high participation and outcome assessment blinded to exposure status reduced likelihood of selection bias. Use of referent group with different occupations results in less similar comparison groups	exposed; higher % male in exposed groups. Duration of exposure and smoking status were not correlated with histology score, therefore confounding not a concern	for mucociliary clearance		survival effect due to irritation effects

Reference	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Size/ estimated power	Comments
Löfstedt et al. (2011) Cross-sectional Study Related study: Westberg et al. (2005) (exposure methods)	Personal sampling over a single 8-hr shift. Formaldehyde concentration, mean (SD), range: 0.051 (0.049) mg/m^3, 0.013−0.190 mg/m^3; 71.4% of exposed <LOD for formaldehyde (<0.02 mg/m^3).	Nasal symptoms and signs; questionnaire; examination by rhinologist blind to exposure status	43 exposed employees at 3 brass foundries producing cores using Hot Box method (90%) Referent: 82 assembly workers and storage workers with no chemical exposure (98%); high participation reduced likelihood of selection bias. Use of referent workers from same companies increased similarities between groups. Possible healthy worker survival selection because of inclusion only of current workers and irritant exposures, but authors said there was no evidence	Evaluated impacts of confounding by exclusion of smokers, females, or asthmatic and allergic subjects from analysis. Other exposures also associated with nasal signs: isocyanic acid (ICA) and methyl isocyanate (MIC) and dust; correlations between co-exposures ranged between −0.08 and 0.65 (except ICA and MIC, r = 0.92); analyses using metric for combined exposure to multiple irritants.	Logistic regression, single-pollutant analyses, OR (95% CI); cut-point for categories of formaldehyde exposed was LOD	69 unexposed, 30 low and 12 high exposure	 Formaldehyde levels among exposed were low (30 of 43 exposed at <LOD). Possible confounding of formaldehyde associations by ICA or MCA, but correlation for pollutant pairs was not reported.

Reference	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Size/ estimated power	Comments
Controlled Human Exposure Studies							
Falk et al. (1994)	Formalin exposure; analytic concentrations, mean: Group 1: 0.021, 0.028, 0.073, 0.174; Group 2: 0.023, 0.029, 0.067, 0.127	Nasal mucosa swelling measured using rhinostereometry (summary of changes for both turbinates)	Double blind exposures, exposure-order stochastically distributed and separated by 2 days.	Within-person comparison	Results presented in graphs	N = 6–7 per group	Overall Confidence **Medium**
Pazdrak et al. (1993)	Test article characterization and exposure generation method not described; clean air followed by 0.5 mg/m^3 formaldehyde	Stage 1 evaluation of symptoms, morphological changes, and biochemical changes in nasal washings. Stage 2 clinical comparison of nasal mucosa by group.	Two-stage, single-blind examination with nonrandom order of exposure assignment.	Within-person comparison	Results presented with statistical analyses	N = 8–11 per group	Overall Confidence **Low**
Andersen and Lundqvist from Andersen and Molhave (1983)	Paraformaldehyde. Dynamic chamber; analytic concentrations; clean air followed by 0.3, 0.5, 1.0, and 2.0 mg/m^3 formaldehyde.	Nasal airflow resistance and nasal mucocilliary flow	Subjects assigned to four groups, each group with four different exposures over four consecutive days, order decided by Latin square design.	Within-person comparison	Results presented with statistical analyses	N = 16	Overall Confidence **Medium**

1

Studies in Animals

In addition to the general factors considered for all toxicology studies of formaldehyde inhalation exposure (see Appendix A.5.1), factors specific to the interpretation of respiratory tract pathology were considered when determining study confidence. These criteria reflect the large database of well-conducted studies, and include: the use of too few test subjects (i.e., a sample size of less than 10 was considered a significant limitation); a failure to report lesion incidence and/or severity; the lumping of multiple lesions (e.g., squamous metaplasia and hyperplasia) together; a failure to report quantitative incidences and/or statistical analyses; the use of insensitive sampling procedures (multiple sections across multiple levels of the respiratory tract were preferred); and use of an exposure duration or follow-up that is likely insensitive for detecting slow-developing lesions (a duration of ≥1 year was preferred). Finally, somewhat in contrast to the available experimental animal studies for other health effect sections, most studies of respiratory pathology used paraformaldehyde or freshly prepared formalin as the test article, although some studies tested commercial formalin. While co-exposure to methanol is a major confounding factor for systemic endpoints, it is less of a concern ("+"; see below) when identifying effects of inhaled formaldehyde on respiratory pathology. Most inhaled methanol bypasses the nose but is readily absorbed in the lungs and distributed systemically. A discussion of the different test articles (i.e., paraformaldehyde, formalin, etc.) used for formaldehyde inhalation studies can be found in Appendix A.5.1. Additional considerations that might influence the interpretation of the usefulness of the studies during the hazard synthesis are noted, including limitations such as the use of only one test concentration or concentrations that are all too high or too low to provide a spectrum of the possible effects, as well as study strengths like very large sample sizes or use of good laboratory practices (GLP); however, this information typically did not affect the study evaluation decisions.

Studies are grouped by exposure duration, and then organized alphabetically by first author. If the conduct of the experimental feature is considered to pose a substantial limitation that is likely to influence the study results, the cell is shaded gray; a "+" is used if potential issues were identified but not expected to have a substantial influence on the interpretation of the experimental results; and a "++" denotes experimental features without limitations that are expected to influence the study results. Specific study details (or lack thereof) that highlight a limitation or uncertainty in answering each of the experimental feature criteria are noted in the table cells. For those experimental features identified as having a substantial limitation likely to influence the study results, the relevant study details are bolded.

Table A-59. Evaluation of controlled inhalation exposure studies examining respiratory pathology in animals

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (unbolded) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations and statistical analysis[d]**	**Overall confidence rating regarding utility for hazard ID[e]**
Criteria relevant to evaluating the experimental details within each experimental feature category	Exposure quality evaluations (see Section B.4.1.2) are summarized (++ = "robust"; + = "adequate"; gray box = poor); relevance of the tested exposure levels is discussed in the hazard synthesis	Sample size provides reasonable power to assess endpoint(s) in question; species, strain, sex, and age relevant to endpoint; no overt systemic toxicity noted or expected	Interpreting the appropriateness, reproducibility, and informativeness of the study design for evaluating respiratory tract pathology. Although no studies designed according to inhalation guidelines were identified, several GLP-compliant studies were identified and are highlighted below	The protocols used to assess respiratory tract pathology are sensitive, complete, discriminating (specific), and biologically sound (reliable); experimenter bias minimized	Statistical methods, group comparisons, & data/variability presentation are appropriate & discerning	Expert judgement based on conclusions from evaluation of the 5 experimental feature categories
Respiratory Pathology—Chronic						
(Appelman et al., 1988) **Rat**	**++**	**+** Small N (N=10)	**++**	**+** *Lesion severity provided for 13-wk but not 52-wk sacrifice*	**++**	**Medium** [small N; limited reporting of lesion severity]
(Dalbey, 1982) **Hamster**	**++**	**++**	**++** Note: single concentration study	**+** *Lesion severities NR*	**++**	**Medium** [failure to report lesion severities]

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (unbolded) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations and statistical analysis[d]**	**Overall confidence rating regarding utility for hazard ID[e]**
(Holmstrom et al., 1989c) **Rat**	**++** Note: high concentration exposure (15.3 mg/m^3-d)	**+** Small N (N=16/group)	**++** Note: single concentration study	**Lesion severities NR; nonstandardized histological characterization makes interpretation of effect difficult**	**Incidence of metaplasia and dysplasia reported together**	**Not Informative** [small N; failure to report lesion severities; incidence of metaplasia and dysplasia reported together]
(Kamata et al., 1997) **Rat**	**+** *Formalin*; methanol concentration was reported and a methanol control was used.	**+** *Inadequate number of animals for interim sacrifices* (N=5)	**++**	**+** *Lesion severities NR;* prevalence of neoplastic lesions complicates assessment of nonneoplastic lesions	**++**	**Medium** [formalin; small N for interim sacrifices; failure to report lesion severities]
(Kerns et al., 1983) **Mouse** See also (Battelle, 1982) and (Swenberg et al., 1980b)	**++**	**+** **Survival to 18 mos was <33% in all groups (N>25)**	**++** Note: data from this study based on a GLP study (1982)	**Lesion severities NR; incidence NR; only three nasal sections (II, III, and V) evaluated**	**++**	**Medium** [somewhat limited sampling, high mortality, and failure to report lesion incidence and severities]

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (unbolded) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations and statistical analysis[d]**	**Overall confidence rating regarding utility for hazard ID[e]**
(Kerns et al., 1983**)** **Rat** See also (Battelle, 1982) and (Swenberg et al., 1980b)	++	+ Transient viral infection at weeks 52–53 was considered unlikely to influence study outcome because of its short course	++ Note: data from this study based on a GLP study (1982)	++ Note: incidence and severity data by nasal section extracted from CIIT (1982)	++	**High** [Note: transient viral infection]
(Monticello et al., 1996**)** **Rat**	++	++	++	**Lesion severities NR; lesion incidence NR**	**Insufficient data to verify magnitude of concentration-response**	**Low** [Failure to report lesion incidence and severities; insufficient data to verity magnitude of concentration-response]
(Sellakumar et al., 1985**)** **Rat** see also (Albert et al., 1982)	+ *Formaldehyde was generated by heating a slurry of paraformaldehyde in paraffin oil* (kerosene), which could cause co-exposure to paraffin oil. [Note: high concentration exposure (18.2 mg/m^3-d)]	++	++ Note: single concentration study	+ *Lesion severities NR*	++	**Medium** [Likely co-exposure to paraffin oil (kerosene); testing at a single high concentration; failure to report lesion severities]

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (unbolded) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations and statistical analysis[d]**	**Overall confidence rating regarding utility for hazard ID[e]**
(Woutersen et al., 1989) **Rat**	++	++	++	+ *Lesion severities NR*; significant incidence of lesions in controls	++ Statistical analyses of lesions NR	**High** [Failure to report lesion severities]
Respiratory Pathology—Subchronic						
(Andersen et al., 2010) **Rat**	++	+ **small N (N=8)**	++	++	+ Data for levels III-V NR; statistical analyses of lesions NR	**Medium** [Small N; data for levels III-V NR]
(Arican et al., 2009) **Rat**	**Analytical method and concentrations NR**	++	++ Note: single concentration study	**Lesion severities NR; lesion incidence NR**	+ *Qualitative descriptions only*	**Not Informative** [Failure to report analytical method and analytical concentrations; failure to report lesion incidence and severities; results described qualitatively]
(Casanova et al., 1994) **Rat**	++	**Small N (N=3)**	++	**Lesion severities NR; lesion incidence NR**	+ *Qualitative descriptions only*	**Not Informative** [Small N; failure to report lesion incidence and severities; results described qualitatively]

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (unbolded) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations and statistical analysis[d]**	**Overall confidence rating regarding utility for hazard ID[e]**
(Coon et al., 1970) **Dog**	**++**	**Small N (N=2)**	**Continuous exposure (22 hrs/d)** Note: single concentration study	**Lesion severity NR; lesion incidence NR**	**+** *Qualitative descriptions only*	**Not Informative** [Small N; single concentration tested; failure to report lesion incidence and severities; results described qualitatively]
(Coon et al., 1970) **Guinea pig**	**++**	**++**	**Continuous exposure (22 hrs/d)** Note: single concentration study	**Lesion severity NR; lesion incidence NR**	**+** *Qualitative descriptions only*	**Not Informative** [Single concentration tested; failure to report lesion incidence and severities; results described qualitatively]
(Coon et al., 1970) **Monkey**	**++**	**Small N (N=3)**	**Continuous exposure (22 hrs/d)** Note: single concentration study	**Lesion severity NR; lesion incidence NR**	**+** *Qualitative descriptions only*	**Not Informative** [Small N; single concentration tested; failure to report lesion incidence and severities; results described qualitatively]

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (unbolded) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations and statistical analysis[d]**	**Overall confidence rating regarding utility for hazard ID[e]**
(Coon et al., 1970) **Rabbit**	++	**Small N (N=3)**	**Continuous exposure (22 hrs/d)** Note: single concentration study	**Lesion severity NR; lesion incidence NR**	+ *Qualitative descriptions only*	**Not Informative** [Small N; single concentration tested; failure to report lesion incidence and severities; results described qualitatively]
(Coon et al., 1970) **Rat**	++	++	**Continuous exposure (22 hrs/d)** Note: single concentration study	**Lesion severity NR; lesion incidence NR**	+ *Qualitative descriptions only*	**Not informative** [Single concentration tested; failure to report lesion incidence and severities; results described qualitatively]
(Feron et al., 1988) **Rat**	++ Note: exposure in the high concentration group was excessive (24.4 mg/m^3-d)	++	++	+ No quantitative interim sacrifice data to inform lesions immediately after exposure	++ Note: recovery period data informs persistence of lesions	**High** [Note: only tested high formaldehyde levels]

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (unbolded) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations and statistical analysis[d]**	**Overall confidence rating regarding utility for hazard ID[e]**
(Horton et al., 1963) **Mouse**	**+** *Analytical concentrations NR* Note: extremely high concentration exposure (200 mg/m^3-d)	**++**	**+** **Early mortality in high exposure group by 11th day of exposure**	*Nose was not examined; lesion severity NR* Note: lesions are of questionable adversity	**++**	**Low** [Analytical concentrations NR; early mortality in the high concentration group, which had an extremely high concentration; nose was not examined; failure to report lesion severity]
(Maronpot et al., 1986) **Mouse**	+ Formalin; methanol concentration was not reported and a methanol control was not used. [Note: high concentration exposure (49.2 mg/m^3)]	**+** *Small N (N=10)*	**++**	**++**	**++**	**Medium** [Formalin; small N]
(Rusch et al., 1983) **Rat**	**++** Note: concentrations tested were very low (0.23–3.6 mg/m^3-d), and unlikely to elicit a response	**++**	**++**	**+** *Lesion severity NR*	**incidence of squamous metaplasia and hyperplasia reported together; data reported for only one nasal section**	**Medium** [Failure to report lesion severity; incidence of squamous metaplasia and hyperplasia reported together; data reported for only one nasal section]

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (unbolded) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations and statistical analysis[d]**	**Overall confidence rating regarding utility for hazard ID[e]**
(Rusch et al., 1983) **Monkey**	++ Note: concentrations tested were very low (0.23–3.6 mg/m^3-d), and unlikely to elicit a response	++	++	+ *Lesion severity NR*	**Incidence of squamous metaplasia and hyperplasia reported together; data reported for only one nasal section**	**Medium** [Failure to report lesion severities; incidence of squamous metaplasia and hyperplasia reported together; data reported for only one nasal section]
(Rusch et al., 1983) **Hamster**	++ Note: concentrations tested were very low (0.23–3.6 mg/m^3-d), and unlikely to elicit a response	++	+ Limited study design: only endpoint evaluated was squamous metaplasia	++	**Specific incidence data NR, so lack of effect could not be verified**	**Medium** [Specific incidence data NR; note: only squamous metaplasia was evaluated]
(Wilmer et al., 1989) **Rat**	+ *Analytical concentrations NR*	++	++	+ *Lesion severity NR*	++	**Medium** [Analytical concentrations NR; failure to report lesion severities]
(Woutersen et al., 1987) **Rat**	++ Note: high concentration exposure (24.4 mg/m^3-d)	++	++	++	++	**High** [Note: the high concentration level was excessive]
(Zwart et al., 1988) **Rat**	++	++	++	+ **Lesion severity NR; lesion incidence incompletely reported**	++	**Medium** [Failure to completely report lesion incidence; severity NR]

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (unbolded) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations and statistical analysis[d]**	**Overall confidence rating regarding utility for hazard ID[e]**
Respiratory Pathology—Short-term						
(Andersen et al., 2008) **Rat**	**+** *≈30% variations in chamber concentrations*	**+** **Small N (N=8)**	**++**	**++**	**+** Statistical analyses of lesions NR	**Medium** [Small N; variation in chamber concentrations]
(Bhalla et al., 1991) **Rat**	**Analytical method and concentrations NR**	**+** *Small N (N=6)*	**+ +** Note: single concentration study	**Lesion severity NR; lesion incidence NR**	**++**	**Not Informative** [Failure to report analytical method and FA concentrations; small N, failure to report lesion incidence and severities]
(Buckley et al., 1984) **Mouse**	+ *Formalin;* methanol concentration was not reported and a methanol control was not used	**++**	**++** Note: single concentration study	**Lesion incidence NR**	**+** Statistical analyses of lesions NR	**Low** [Formalin; failure to report lesion incidence]
(Cassee and Feron, 1994) **Rat**	**++**	**++**	**++** Note: single concentration study	**+** *Incidence and severity of hyperplasia and metaplasia reported together*	**+** Statistical analyses of lesions NR	**Medium** [Incidence and severities of hyperplasia and metaplasia were reported together]
(Cassee et al., 1996b) **Rat**	**++**	**+** *Small N (N=6)*	**++**	**+** *Data NR for 7.9 mg/m^3 group*	**+** Statistical analyses of lesions NR	**Medium** [Small N, failure to report data for the 7.0 mg/m^3 group]

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (unbolded) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations and statistical analysis[d]**	**Overall confidence rating regarding utility for hazard ID[e]**
(Chang et al., 1983) **Rat**	++	**Sample size N unclear**	Note: single concentration study; this study measured reflex bradypnea	**Lesion severity NR; lesion incidence NR**	+ Statistical analyses of lesions NR	**Low** [Sample size unclear, failure to report lesion incidence and severity]
(Chang et al., 1983) **Mouse**	++	**Sample size N unclear**	Note: single concentration study; this study measured reflex bradypnea	**Lesion severity NR; lesion incidence NR**	+ Statistical analyses of lesions NR	**Low** [Sample size unclear, failure to report lesion incidence and severity]
(Ionescu et al., 1978) **Rabbit**	**Test article characterization NR; analytical concentrations NR; formaldehyde generation method NR**	**Test subject strain and number NR**	++ Note: single concentration study	**Lesion severity NR; lesion incidence NR**	++	**Not Informative** [Analytical concentrations NR; test article characterization NR; FA generation method NR; test subject strain and number NR; failure to report lesion incidence and severity]
(Kamata et al., 1996b) **Rat**	+ *Formalin*; no methanol control or concentration was reported. [Note: high concentration exposure (179.1 mg/m^3)]	+ *Small N (N=5) for histo-pathology*	++	**Lesion severity NR; lesion incidence NR**	+ Statistical analyses of lesions NR	**Low** [Formalin; small N for histopathology; failure to report lesion incidence and severities]

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (unbolded) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations and statistical analysis[d]**	**Overall confidence rating regarding utility for hazard ID[e]**
(Kuper et al., 2011) **Rat**	**+** Appears to be freshly made formalin; although formaldehyde generation method NR	**+** *Small N (N=8)*	**++** Note: GLP-compliant study	**++**	**++**	**High** [Small N]
(Kuper et al., 2011) **Mouse**	**+** Appears to be freshly made formalin; although formaldehyde generation method NR	**+** *Small N (N=6)*	**++** Note: GLP-compliant study	**++**	**++**	**High** [Small N]
(Lima et al., 2015) **Rat**	**Test article characterization NR; concentrations NR-likely high levels**	**+** *Small N (N=7)*; males only	**Short (20 min × 3) daily exposures**; **controls did not appear to be chamber exposed.** Note: 5 d exposure	**Lesion severity NR; lesion incidence (nonmorphometric analyses) NR** Note: randomized, but blinding NR	**+** Statistical analyses of lesions NR	**Not Informative** [Failure to characterize the test article and report levels; short periodicity; lesion data NR]
(Monteiro-Riviere and Popp, 1986) **Rat**	**++**	**+** *Small N (N=5; note: only 3/ treated group examined in "detail")*	**++**	**Lesion severity NR; lesion incidence NR**	**+** Statistical analyses of lesions NR	**Medium** [Small N; lesion incidence and severity NR]
(Monticello et al., 1989) **Monkey**	**+** *Analytical concentrations NR*	**++**	**++** Note: single concentration study	**Lesion severity NR; lesion incidence NR**	**++**	**Medium** [Analytical concentrations NR; lesion incidence and severity NR]

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (unbolded) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations and statistical analysis[d]**	**Overall confidence rating regarding utility for hazard ID[e]**
(Murta et al., 2016) **Rat**	**Test article characterization NR; concentrations NR-likely high levels**	**+** *Small N (N=7)*; males only	**Short (20 min × 3) daily exposures** note: 5 d exposure	**Lesion severity NR; lesion incidence (nonmorphometric analyses) NR** Note: randomized, but blinding NR	**+** Statistical analyses of lesions NR	**Not Informative** [Failure to characterize the test article and report levels; short periodicity; lesion data NR]
(Morgan et al., 2017) **Mouse**	**+** *Analytic concentrations NR*	**++** Note: "randomly assigned"; Males only; ≈25 mice/ group; genetically modified (Trp53+/-)	**++** Note: 8 wk exposure duration with 32 wk follow up was not a notable issue for these outcomes as numerous lesions found	**+** *Blinding NR; only 3 nasal sections evaluated (and 1 larynx)*	**+** Statistical analyses of lesions NR	**Medium** [limited sampling and minor reporting limitations]
(Reuzel et al., 1990) **Rat**	**++**	**++**	**++**	**++**	**+** Statistical analyses of lesions NR	**High**
(Schreiber et al., 1979) **Hamster**	**Test article characterization NR; analytical concentrations NR; formaldehyde generation method NR** Note: high concentration exposure (307.5 mg/m^3)	**+** *Small N (N=3 to 5)*	**++** Note: single concentration study	**Lesion severity NR; lesion incidence NR**	**+** Statistical analyses of lesions NR	**Not Informative** [Failure to characterize the test article, describe the generation method, and report analytical concentrations; failure to report lesion incidence and severities]

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (unbolded) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations and statistical analysis[d]**	**Overall confidence rating regarding utility for hazard ID[e]**
(Speit et al., 2011b) **Rat**	**+** Formalin; methanol concentration was not reported and a methanol control was not used	**+** *Small N (N=6)*	**++**	**++**	**++**	**Medium** [Small N; formalin]
(Wilmer et al., 1987) **Rat**	**+** *Analytical concentrations NR*	**++**	**++**	**Lesion severity NR; lesion incidence NR**	**++** Note: intermittent versus continuous exposures compared	**Medium** [Analytical concentrations NR; failure to report lesion incidence and severities]
(Yorgancilar et al., 2012) **Rat**	**Test article characterization NR; analytical concentrations NR; formaldehyde generation method NR**	**+** **Small N (N=8)**	**+** Note: single concentration study	**Lesion severity NR; lesion incidence NR**	**+** Statistical analyses of lesions NR	**Not Informative** [Failure to characterize test article; failure to report analytical concentrations and generation method; small N; failure to report lesion incidence and severities]

NR = not reported; N/A = not applicable.

[a]Gray = inadequate N (N= 1 or 2) or multiple less essential study details (e.g., sex, strain) NR; + = inadequate N (e.g., N= ≥2 to ≤10) or individual less essential study details NR; ++ = adequate N (using guidance from OECD TG 452 and TG 413: chronic: ≥20 animals/sex/group; subchronic: 10 animals/sex/group, respectively).

[b]Gray = test protocols for assessing endpoints could not be evaluated or had critical flaws, timing of exposures expected to compromise the integrity of the protocols, protocols completely irrelevant to human exposure; + = informative components of the protocol were NR/insufficiently assessed, limited human relevance or single concentration study; ++ = protocol considered relevant to human exposure.

[c]Gray = uncontrolled variables are expected to confound the results or lack of reporting for lesion incidence and severity; + = limited information provided for observed lesions (i.e., incidence and/or severity) uncontrolled variables may significantly influence results; ++ = adequate reporting of data, no potential confounding identified.

[d]Gray = failure to report a sufficient amount of data to verify results; + = failure to report statistical analyses; ++ = adequate reporting of data.

[e]Designation for Utility for Hazard ID (i.e., confidence) based on EPA judgment regarding the five evaluated criteria, with multiple impactful "gray" categories generally leading to a designation of "not informative."

Table A-60. Evaluation of controlled inhalation exposure studies examining cell proliferation and mucociliary function in animals

	Experimental Feature Categories *The study details leading to identification of major (**bolded**) or minor (unbolded) experimental feature limitations are indicated.*					
	Exposure Quality	**Test Subjects[a]**	**Study Design[b]**	**Endpoint Evaluation[c]**	**Data Considerations & Statistical Analysis[d]**	**Overall Confidence Rating Regarding Utility for Hazard ID[e]**
Criteria relevant to evaluating the experimental details within each experimental feature category	Exposure quality evaluations (see B.4.1.2) are summarized (++ = "robust"; + = "adequate"; gray box = poor); relevance of the tested exposure levels is discussed in the hazard synthesis	Sample size provides reasonable power to assess endpoint(s) in question; species, strain, sex, and age relevant to endpoint; no overt systemic toxicity noted or expected	Interpreting the appropriateness, reproducibility, and informativeness of the study design for evaluating respiratory tract pathology. Although no studies designed according to inhalation guidelines were identified, several GLP-compliant studies were identified and are highlighted below	The protocols used to assess respiratory tract pathology are sensitive, complete, discriminating (specific), and biologically sound (reliable); experimenter bias minimized	Statistical methods, group comparisons, and data/variability presentation are appropriate and discerning	Expert judgement based on conclusions from evaluation of the 5 experimental feature categories
Cell Proliferation						

	Experimental Feature Categories *The study details leading to identification of major (**bolded**) or minor (unbolded) experimental feature limitations are indicated.*					
	Exposure Quality	**Test Subjects[a]**	**Study Design[b]**	**Endpoint Evaluation[c]**	**Data Considerations & Statistical Analysis[d]**	**Overall Confidence Rating Regarding Utility for Hazard ID[e]**
(Andersen et al., 2008) **Rat**	**+** **≈30% variations in atmospheres**	**++**	**++**	**++**	**++**	**High**
(Andersen et al., 2010) **Rat**	**++**	**+** **Variable sample size (N=1 to 8)**	**++**	**++**	**++**	**High**
(Casanova et al., 1994) **Rat**	**++**	**++**	**Relevance of exposure scenario unclear (Note: nasal regions selected for analysis may not be relevant to humans)**	**++**	**++**	**Medium**
(Cassee and Feron, 1994) **Rat**	**++**	**+** **Number of cells analyzed NR**	**++** Note: single concentration study	**++**	**++** **Qualitative data only**	**Medium**
(Cassee et al., 1996b) **Rat**	**++**	**+** **Small N (N=3 to 5)**	**++**	**+** **Data for 7.9 mg/m^3 NR**	**++**	**High**
(Chang et al., 1983) **Rat**	**++**	**+** **Variable sample size (N=4 to 9)**	**Unclear description of study design** Note: single concentration study	**++**	**++**	**Medium**
(Chang et al., 1983) **Mouse**	**++**	**+** **Variable sample size (N=4 to 10)**	**Unclear description of study design** Note: single concentration study	**++**	**++**	**Medium**

	Experimental Feature Categories *The study details leading to identification of major (**bolded**) or minor (unbolded) experimental feature limitations are indicated.*					
	Exposure Quality	**Test Subjects[a]**	**Study Design[b]**	**Endpoint Evaluation[c]**	**Data Considerations & Statistical Analysis[d]**	**Overall Confidence Rating Regarding Utility for Hazard ID[e]**
(Kuper et al., 2011) **Rat**	++ **Formaldehyde generation method NR**	++	++ Note: GLP-compliant study	++	++	**High**
(Kuper et al., 2011) **Mouse**	++ **Formaldehyde generation method NR**	++	++ Note: GLP-compliant study	++	++	**High**
(Meng et al., 2010) **Rat**	+ **Analytical concentrations NR**	++	++	++	++	**High**
(Monticello et al., 1991) **Rat**	++	+ **Variable sample size (N=4 to 6)**	++	++	++	**High**
(Monticello et al., 1989) **Monkey**	+ **Analytical concentrations NR**	++	+ Note: single concentration study	+ **Qualitative data only for nasal region**	++	**Medium**
(Monticello et al., 1996) **Rat**	++	+ **Variable sample size (N=3 to 8)**	+ **Nonstandard selection of nasal regions; Note: regions may not be relevant to humans**	++	+ **Statistical analyses of cell proliferation NR**	**Medium**
(Reuzel et al., 1990) **Rat**	++	++	++	++	+ +	**High**

	Experimental Feature Categories *The study details leading to identification of major (**bolded**) or minor (unbolded) experimental feature limitations are indicated.*					
	Exposure Quality	**Test Subjects[a]**	**Study Design[b]**	**Endpoint Evaluation[c]**	**Data Considerations & Statistical Analysis[d]**	**Overall Confidence Rating Regarding Utility for Hazard ID[e]**
(Roemer et al., 1993) **Rat**	++	++	++	++	++	**High**
(Speit et al., 2011b) **Rat**	+ **Formalin exposure**; no methanol controls and concentration NR	++	++	++	++	**Medium**
(Wilmer et al., 1987) **Rat**	+ **Analytical concentrations NR**	**Small and variable sample size (N=1 to 3)**	++	++	++	**Medium**
(Wilmer et al., 1989) **Rat**	+ **Analytical concentrations NR**	++	++	++	++	**High**
(Feron et al., 1987) **Rat**	++ Note: high concentration exposure (24.4 mg/m^3-d)	**Small N (N=2)**	++	++	+ **Statistical analyses of cell proliferation NR**	**Medium**
(Zwart et al., 1988) **Rat**	++	++	++	++	+ **Cell proliferation data not readily accessible from graphic form**	**High**
Mucociliary Function						

	Experimental Feature Categories *The study details leading to identification of major (**bolded**) or minor (unbolded) experimental feature limitations are indicated.*					
	Exposure Quality	**Test Subjects[a]**	**Study Design[b]**	**Endpoint Evaluation[c]**	**Data Considerations & Statistical Analysis[d]**	**Overall Confidence Rating Regarding Utility for Hazard ID[e]**
(Fló-Neyret et al., 2001) **Frog**	**Not an inhalation study. Exposure based on immersion into formaldehyde solution (i.e., formalin)**	**+ frogs**	**Ex vivo amphibian study; experiments carried out three days after sacrifice; mucus removed from palate during preparation and returned to palate for testing**	**++**	**++**	**Not Informative**
(Morgan et al., 1984) **Frog**	**+ Analytical concentrations within 20% of nominal**	**+ frogs**	**Ex vivo amphibian study; method of sacrifice (anesthesia) and palate harvest NR**	**+ Inter-animal variation observed at several concentrations**	**++**	**Low**
(Morgan et al., 1986a) **Rat**	**++**	**++**	**++** Note: mucociliary function assessed using dissected nasal cavities	**++**	**+ Statistical analyses of mucociliary function data NR**	**High**
(Morgan et al., 1986c) **Rat**	**++**	**++**	**++** Note: mucociliary function assessed using dissected nasal cavities	**++**	**+ Statistical analyses of mucociliary function data NR**	**High**

NR = not reported; N/A = not applicable.

[a]Gray = inadequate N (N= 1 or 2) or multiple less essential study details (e.g., sex, strain) NR; + = inadequate N (e.g., N= ≥2 to ≤10) or individual less essential study details NR; ++ = adequate N.

[b]Gray = Test protocols for assessing endpoints could not be evaluated or had critical flaws, timing of exposures expected to compromise the integrity of the protocols, protocols completely irrelevant to human exposure; + = informative components of the protocol were NR/insufficiently assessed, limited human relevance or single concentration study; ++ = protocol considered relevant to human exposure.

[c]Gray = uncontrolled variables are expected to confound the results; + = limited information provided for observations (e.g., qualitative data) or uncontrolled variables may significantly influence results; ++ = adequate reporting of data, no potential confounding identified.
[d]Gray = failure to report a sufficient amount of data to verify results; + = failure to report statistical analyses; ++ = adequate reporting of data.
[e]Designation for Utility for Hazard ID based on EPA judgment and the following criteria: gray = the presence of generally >2 gray boxes in the study feature categories; low = failure in 2 categories; medium = failure in 1 category; high = no category failures; the presence of multiple +'s may demote tier level.

Supporting Material for Hazard Analyses of Respiratory Tract Pathology

Supplementary materials relevant to evaluating the evidence for respiratory tract pathology are described below. Cell proliferation and mucociliary function studies, which inform the potential mode(s) of action for the induction of respiratory tract pathology following formaldehyde inhalation, are described in Appendix A.5.6.

Supportive short-term respiratory tract pathology studies in experimental animals

Due to the abundance of high-quality, longer duration exposure studies on respiratory tract effects in experimental animals, the results of supportive *medium* and *high confidence* short-term studies that did not provide information that was unexamined or inadequately examined in the longer term studies (i.e., species differences; the relative contribution of concentration and duration to lesion development) are summarized below (note: the details of *low confidence* animal studies are not described for respiratory pathology owing to the large number of *high* and *medium confidence* studies available).

Table A-61. Supportive short-term respiratory pathology studies in animals

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td colspan="2">RAT</td></tr>
<tr><td colspan="2">High Confidence</td></tr>
<tr><td>Reuzel et al. (1990)
Wistar rats; male; 10/group.
Exposure: Rats were exposed to FA in dynamic whole-body chambers 22 hrs/d for 3 d.
Test article: Paraformaldehyde.
Actual concentrations were 0, 0.37 (±0.01), 1.4 (±0.0), and 3.8 (±0.1) mg/m^3.[1]
This study also evaluated the combined effects of ozone and FA mixtures on nasal epithelium. Data presented here in the Results column are for FA-only exposed rats.
Histopathologic evaluation of the respiratory tract included 6 standard sections of the nose.</td><td>
<table>
<tr><td></td><td colspan="6">Concentration of FA</td></tr>
<tr><td></td><td colspan="2">0 mg/m^3</td><td colspan="2">0.37 mg/m^3</td><td colspan="2">1.4 mg/m^3</td></tr>
<tr><td></td><td>II[a]</td><td>III[a]</td><td>II</td><td>III</td><td>II</td><td>III</td></tr>
<tr><td colspan="7">Disarrangement/loss of cilia without hyper/metaplasia</td></tr>
<tr><td>Minimal to slight</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/9</td><td>0/9</td></tr>
<tr><td>Moderate</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/9</td><td>0/9</td></tr>
<tr><td colspan="7">Disarrangement/loss of cilia with hyper/metaplasia</td></tr>
<tr><td>Minimal to slight</td><td>0/10</td><td>0/10</td><td>1/10</td><td>0/10</td><td>2/9</td><td>0/9</td></tr>
<tr><td>Moderate</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/9</td><td>0/9</td></tr>
<tr><td>Marked</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/9</td><td>0/9</td></tr>
<tr><td colspan="7">Keratinization</td></tr>
<tr><td>Minimal to slight</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/9</td><td>0/9</td></tr>
<tr><td>Moderate</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/9</td><td>0/9</td></tr>
<tr><td colspan="7">Rhinitis</td></tr>
<tr><td>Minimal to slight</td><td>0/10</td><td>0/10</td><td>2/10</td><td>0/10</td><td>1/9</td><td>0/9</td></tr>
<tr><td>Moderate</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/9</td><td>0/9</td></tr>
</table>
[a]Level in the nose examined.
<table>
<tr><td></td><td colspan="4">Concentration of FA</td></tr>
<tr><td></td><td colspan="2">0 mg/m^3</td><td colspan="2">3.8 mg/m^3</td></tr>
<tr><td></td><td>II[a]</td><td>III[a]</td><td>II</td><td>III</td></tr>
<tr><td>Disarrangement/loss of cilia without hyper/metaplasia</td></tr>
</table>
</td></tr>
</table>

Reference and study design	Results

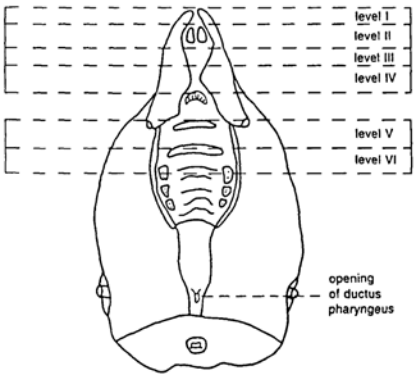


Figure 1 from Reuzel et al. (1990) depicting cross levels of the rat nose evaluated for histopathological lesions.

Main limitations: No major limitations.

Minimal to slight	0/10	0/10	0/10	0/10
Moderate	0/10	0/10	0/10	0/10
Disarrangement/loss of cilia with hyper/metaplasia				
Minimal to slight	0/10	0/10	7/10	3/10
Moderate	0/10	0/10	3/10	5/10
Marked	0/10	0/10	2/10	0/10
Keratinization				
Minimal to slight	0/10	0/10	7/10	0/10
Moderate	0/10	0/10	1/10	0/10
Rhinitis				
Minimal to slight	0/10	0/10	0/10	0/10
Moderate	0/10	0/10	0/10	0/10

[a]Level in the nose examined.

Histopathological changes for Level I not reported.
Histopathological changes for Levels IV, V, and VI reported together. Only change observed was minimal to slight rhinitis in rats (4/10) exposed to 3.8 mg/m^3 FA.

Medium Confidence

Andersen et al. (2008)
Fischer 344 rats; male; 8/group.
Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for up to 3 wks. Rats sacrificed at end of single 6-hr exposure (day 1), 18 hrs after single 6-hr exposure (day 1 recovery), at end of 5 d of exposure (day 5), at end of 6 d of exposure (day 6), 18 hrs after 6 d of exposure (day 6 recovery), and at end of 15 d of exposure (day 15).
Test article: Paraformaldehyde.
Actual concentrations were determined on a daily basis and reported in the **Results** column. Target concentrations were 0, 0.9, 2.5, 7.4, and 18.5 mg/m^3.[1]

This study also evaluated the effects of a single FA instillation (40 µL, 400 mM per nostril). Data presented here in the **Results** column are for inhalation exposures.

Histopathologic evaluation of the respiratory tract included nasal sections at levels I (front of nose), II (anterior lateral meatus, anterior septum, medial aspect maxilloturbinate), and III (posterior lateral meatus, posterior septum).

Target and Actual FA Concentrations[a]

Target concentration (mg/m^3)	Day 1 (mg/m^3)	Day 5 (mg/m^3)	Day 6 (mg/m^3)	Day 15 (mg/m^3)
0	0±0	0±0	0±0	0±0
0.9	0.74±0.23	0.79±0.15	0.75±0.16	0.7±0.11
2.5	2.08±0.46	2.14±0.43	2.26±0.49	2.2±0.31
7.4	5.83±1.73	6.43±0.76	6.00±1.25	6.14±0.97
18.5	17.7±5.7	NA	NA	NA

[a]Daily means ± SD.

Histopathology Incidence

	FA (mg/m^3)							
	0	0.9		2.5		7.4		
Time point	InI[a]	InI	EH	InI	EH	InI	EH	SM
Day 1	0[b]	1	0	6	0	8	0	0
Day 1 R[c]	4	2	1	1	3	7	8	0
Day 5	1	1	0	5	3	8	8	7
Day 6	5	2	0	4	1	7	8	0
Day 6 R	6	1	0	3	2	7	8	0
Day 15	3	1	0	0	2	5	7	0

0 ppm: EH and SM were ND; 0.7 ppm: SM was ND; 2 ppm SM was ND
[a]InI = inflammatory infiltrate; EH = epithelial hyperplasia; SM = squamous metaplasia.
[b]Number of animals with the lesion (n = 8).
[c]Recovery group.

Histopathological Incidence

	FA (mg/m^3)	
	0	18.5

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td>Main limitations: small sample size; somewhat high variability in chamber concentrations.</td><td>
<table>
<tr><td></td><td></td><td colspan="3">Level I</td><td colspan="3">Level II</td></tr>
<tr><td>Time point</td><td>InI[a]</td><td>InI</td><td>UcL</td><td>EH</td><td>InI</td><td>UcL</td><td>EH</td></tr>
<tr><td>Day 1</td><td>0[b]</td><td>8</td><td>NR</td><td>NR</td><td>7</td><td>2</td><td>1</td></tr>
</table>
0 ppm: UcL was NR, EH was ND
[a]InI = inflammatory infiltrate; UcL = ulcerative lesions; EH = epithelial hyperplasia.
[b]Number of animals with the lesion (n = 8).
</td></tr>
<tr><td>Cassee and Feron (1994)
Wistar rats; male; 20/group.
Exposure: Rats were exposed in dynamic nose-only chambers for 3 d (6 consecutive 12-hr periods of 8 hrs of exposure to FA followed by 4 hrs of nonexposure). Rats sacrificed immediately (i.e., within 30 min) after last exposure.
Test article: Paraformaldehyde.
Actual concentrations were 0 and 4.4 (SE ± 0.1) mg/m^3 FA.
Histopathologic evaluation of the respiratory tract included standard cross sections of the head (see cross sections in Reuzel et al. (1990).

Main limitations: hyperplasia and metaplasia were reported together.

This study also evaluated the nasal changes induced by exposures to ozone alone and FA and ozone. Data presented here in the Results column are for FA-only exposures.</td><td>
<table>
<tr><td></td><td colspan="2">Controls</td><td colspan="2">FA</td></tr>
<tr><td>Type of lesions</td><td>II[a]</td><td>III[a]</td><td>II</td><td>III</td></tr>
<tr><td colspan="5">Disarrangement, flattening and slight basal cell hyperplasia</td></tr>
<tr><td>Minimal</td><td>0/5</td><td>1/5</td><td>0/5</td><td>0/5</td></tr>
<tr><td>Slight</td><td>0/5</td><td>0/5</td><td>0/5</td><td>0/5</td></tr>
<tr><td>Frank necrosis</td><td>0/5</td><td>0/5</td><td>5/5</td><td>5/5</td></tr>
<tr><td colspan="5">Hyperplasia accompanied by squamous metaplasia</td></tr>
<tr><td>Slight</td><td>0/5</td><td>0/5</td><td>2/5</td><td>3/5</td></tr>
<tr><td>Moderate</td><td>0/5</td><td>0/5</td><td>2/5</td><td>2/5</td></tr>
<tr><td>Marked</td><td>0/5</td><td>0/5</td><td>1/5</td><td>0/5</td></tr>
<tr><td colspan="5">Rhinitis</td></tr>
<tr><td>Slight[b]</td><td>0/5</td><td>0/5</td><td>0/5</td><td>0/5</td></tr>
<tr><td>Moderate</td><td>0/5</td><td>0/5</td><td>0/5</td><td>4/5</td></tr>
<tr><td>Marked</td><td>0/5</td><td>0/5</td><td>5/5</td><td>1/5</td></tr>
</table>
[a]Standard cross section level II and III.
[b]Influx of neutrophils mainly observed.
</td></tr>
<tr><td>Cassee et al. (1996b)
Wistar rats; male; number of animals per group varied but are reported in the Results column.
Exposure: Rats were exposed to FA in dynamic nose-only chambers 6 hrs/d for 1 or 3 d. Rats sacrificed immediately after last exposure.
Test article: Paraformaldehyde.
Actual concentrations were 0, 1.2, 3.9, and 7.9 mg/m^3.[1]

Histopathologic evaluation of the respiratory tract included standard cross sections at levels II, III, and/or IV of the nose (see Reuzel et al. (1990) for cross-sectional levels).</td><td>
1-day exposure: no treatment-related histopathological nasal lesions observed

Histopathological changes from 3 days of exposure[a]
<table>
<tr><td></td><td colspan="3">FA (mg/m^3)</td></tr>
<tr><td>Site, type, degree, and incidence of lesions</td><td>0</td><td>1.2</td><td>3.9</td></tr>
<tr><td>Number of noses examined</td><td>19</td><td>5</td><td>6</td></tr>
<tr><td>Disarrangement, necrosis, thickening, and desquamation of respiratory/transitional epithelium[b]</td><td></td><td></td><td></td></tr>
<tr><td>Slight (mainly disarrangement)</td><td>0</td><td>0</td><td>3</td></tr>
<tr><td>Moderate</td><td>0</td><td>0</td><td>2</td></tr>
<tr><td>Severe (extensive)</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Basal cell hyperplasia and/or increased number of mitotic figures in respiratory/transitional epithelium</td><td></td><td></td><td></td></tr>
<tr><td>Slight (focal)</td><td>0</td><td>0</td><td>4</td></tr>
<tr><td>Moderate</td><td>0</td><td>0</td><td>2</td></tr>
<tr><td>Severe (extensive)</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Increased incidence of “single-cell necrosis” in olfactory epithelium[c]</td><td></td><td></td><td></td></tr>
</table>
</td></tr>
</table>

Reference and study design	Results

Main limitations: small N; failure to report data for the 7.9 mg/m^3 group.

This study also evaluated the combined effects of FA, acetaldehyde, and acrolein on nasal epithelium. Data presented here in the **Results** column are for FA-only exposed rats.

A few necrotic cells	0	0	0
A moderate number of necrotic cells	0	0	0
Many necrotic cells	0	0	0
Atrophy of olfactory epithelium			
Slight (mainly disarrangement)	0	0	0
Moderate (focal)	0	0	0
Severe (extensive)	0	0	0
Rhinitis			
Slight	2	1	0
Moderate	1	0	0
Severe	0	0	0

[a]Data for 7.9 mg/m^3 group NR.
[b]Changes observed at levels II and III.
[c]Changes observed at levels III and IV.

Monteiro-Riviere and Popp (1986)
Fischer 344 rats; male; 3–5/group.
Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/d for either 1, 2, or 4 d. Interim sacrifices were performed either immediately or 18 hrs after last exposure.
Test article: Paraformaldehyde.
Actual concentrations were 0, 0.6 (±0.1), 2.7 (±0.4), 7.3 (±0.1), and 18.2 (±0.4) mg/m^3.[1]
Histopathologic evaluation of the respiratory tract included transverse sections of the skull that contained the dorsal nasal concha, lateral wall, and ventral nasal concha.

Main limitations: small N; (note: only 3 of 5 rats/ treatment group were evaluated in "detail"); failed to report lesion incidence and severity

Cellular occurrence of ultrastructure lesion[a,b]	7.3 mg/m^3[c]	7.3 mg/m^3 (1-day)[d]	7.3 mg/m^3 (2-day)	7.3 mg/m^3 (4-day)
Cytoplasmic vacuoles	ALL	ALL		NC
Autophagic vacuoles	BA	BA		BA, CU, NC
Loss of microvilli	CI	CI	CI	CI, CU, BR
Hypertrophy		CI, GO	CI, GO	CI, GO
SER in apical region		NC		NC
Intracytoplasmic lumen			CI	
Mitochondrial swelling				CI, BR
Neutrophils	+	+	+	
Intercellular edema		+	+	
Ciliated mucous cells			+	+
Nonkeratinized squamous cells				+

[a]Abbreviations: BA, basal cells; CI, ciliated cells; CU, cuboidal cells; BR, brush cells; NC, nonciliated columnar cells; GO, goblet cells; SER, smooth endoplasmic reticulum; ALL, all cell types; +, indicates presence. Nucleolar segregation, pyknotic nuclei, and internalized cilia not observed.
[b]These lesions were not observed at 0.6 mg/m^3 (1 or 4 d exposure) or 2.7 mg/m^3 (1 or 4 d exposure) FA.
[c]Rats in this group were immediately sacrifice after exposure.
[d]Number of days of exposure, rats sacrificed 18 hrs later.

Cellular occurrence of ultrastructure lesion[a,b]	18.2 mg/m^3 (1-d)[c]	18.2 mg/m^3 (2-d)
Cytoplasmic vacuoles	CU, NC	NC
Autophagic vacuoles	BA, CI, CU, NC	BA, CU, NC
Loss of microvilli	BA, CI, CU	CI, CU, NC

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td></td><td>
<table>
<tr><td>SER in apical region</td><td>NC</td><td>NC</td></tr>
<tr><td>Nucleolar segregation</td><td>BA, CU</td><td>BA, CU</td></tr>
<tr><td>Pyknotic nuclei</td><td>CU</td><td>CI</td></tr>
<tr><td>Internalized cilia</td><td>CI</td><td>CI</td></tr>
<tr><td>Neutrophils</td><td>+</td><td></td></tr>
<tr><td>Intercellular edema</td><td>+</td><td></td></tr>
<tr><td>Nonkeratinized squamous cells</td><td>+</td><td>+</td></tr>
</table>
[a]Abbreviations: BA, basal cells; CI, ciliated cells; CU, cuboidal cells; BR, brush cells; NC, nonciliated columnar cells; GO, goblet cells; SER, smooth endoplasmic reticulum; ALL, all cell types; +, indicates presence. Hypertrophy, Intracytoplasmic lumen, mitochondrial swelling, and ciliated mucous cells not observed.
[b]These lesions were not observed at 0.6 mg/m^3 (1 or 4 d exposure) or 2.7 mg/m^3 (1 or 4 d exposure) FA.
[c]Number of days of exposure, rats sacrificed 18 hrs later.
</td></tr>
<tr><td>Speit et al. (2011b)
Fischer 344 rats; males; 6/group.
Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for 4 wks.
Test article: Formalin (methanol concentration NR).
Actual concentrations were 0, 0.63 (±0.6), 1.23 (±0.14), 2.48 (±0.18), 7.53 (±0.42), 12.3 (±0.48), 18.4 (±0.06) mg/m^3.[1]

Histopathologic evaluation of the respiratory tract included 4 levels of the nasal cavity: I (nasal septum, lateral meatus [wall], maxilloturbinate, nasoturbinate), II (nasal septum, lateral meatus [wall]), and III and IV (nasopharynx).

Main limitations: Formalin; small N</td><td>
No FA-related histological changes observed in levels I–IV of rats exposed to 0.63, 1.23, 2.48, and 7.53 mg/m^3.

Histopathological analysis of nasal lesions after 4 wks
<table>
<tr><td></td><td colspan="4">Incidence and grading of findings[a]</td></tr>
<tr><td></td><td></td><td colspan="3">FA (mg/m^3)</td></tr>
<tr><td></td><td>Grade[b]</td><td>0</td><td>12.3</td><td>18.4</td></tr>
<tr><td>Level I</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Metaplasia, squamous</td><td>1</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td></td><td>2</td><td>0</td><td>5</td><td>0</td></tr>
<tr><td></td><td>3</td><td>0</td><td>0</td><td>4</td></tr>
<tr><td></td><td>4</td><td>0</td><td>0</td><td>2</td></tr>
<tr><td>Degeneration, (multi) focal</td><td>2</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td></td><td>3</td><td>0</td><td>0</td><td>3</td></tr>
<tr><td></td><td>4</td><td>0</td><td>0</td><td>2</td></tr>
<tr><td>Inflammation, (multi) focal</td><td>2</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td></td><td>3</td><td>0</td><td>0</td><td>4</td></tr>
<tr><td>Level II</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Metaplasia, squamous</td><td>2</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td></td><td>3</td><td>0</td><td>0</td><td>5</td></tr>
<tr><td>Degeneration, (multi) focal</td><td>1</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td></td><td>2</td><td>0</td><td>0</td><td>2</td></tr>
<tr><td></td><td>3</td><td>0</td><td>0</td><td>3</td></tr>
<tr><td>Inflammation, (multi) focal</td><td>2</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>Level III</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Metaplasia, transitional</td><td>1</td><td>0</td><td>0</td><td>4</td></tr>
<tr><td></td><td>2</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>Level IV</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Metaplasia, transitional</td><td>1</td><td>0</td><td>0</td><td>2</td></tr>
<tr><td></td><td>2</td><td>0</td><td>0</td><td>3</td></tr>
</table>
[a]Number of animal with lesions (6 analyzed per group).
[b]1 = minimal; 2 = slight; 3 = moderate; 4 = severe/marked.
</td></tr>
</table>

[1]Study authors originally reported FA concentrations in ppm. These values were converted based on 1 ppm = 1.23 mg/m^3, assuming 25°C and 760 mm Hg.
Abbreviations: **FA**—Formaldehyde; **NA**—Not applicable; **ND**—Not detected; **NR**—Not reported; **SD**—Standard deviation; **SE**—Standard error of the mean.

A.5.6. Mechanistic Evidence Related to Potential Noncancer Respiratory Health Effects

Note: Large sections of this analysis are redundant to synthesis text, figures, and tables presented in the Toxicological Review and Assessment Overview. However, the entirety of the analyses and discussion is included below to contextualize the conclusions described in the Toxicological Review with the appropriate methodological considerations, supporting analyses, and other information of potential interest.

Organization and Methods

This evaluation provides an integrated discussion characterizing potential relationships between the mechanistic changes observed following formaldehyde inhalation in the context of potential respiratory effects, but it does not attempt to explicitly define a single mode of action.

Literature search strategy

Through 2017, studies were identified through one of two strategies, namely, identification of studies relevant to mechanisms for potential respiratory effects during systematic searches for health hazard-specific toxicity information (see Appendix Sections A.5.2–A.5.5), or through an independent systematic literature search focused on inflammation- and immune-related changes (discussed here). This latter effort was undertaken to identify mechanistic information related to changes in the respiratory tract, blood, and lymphoid tissues that might not have been captured by health effect-specific systematic searches. The comprehensiveness of this strategy was compared against citations in the recent National Academy of Sciences review of the National Toxicology Program Report on Carcinogens (NRC, 2014), and some supportive information from that report is noted in this analysis[17] (i.e., hematological findings from four foreign language studies: (Tong et al., 2007; Yang, 2007; Cheng et al., 2004; Tang and Zhang, 2003). Given the breadth of this topic, this section uses a hierarchical approach to screen, sort, and distill information from over 10,000 references identified across these searches. Thus, additional steps were taken to focus this analysis on the most influential information. In addition to criteria identifying studies as relevant to assessing potential respiratory system changes, studies that failed to report a specific estimate of formaldehyde exposure (e.g., concentration, duration) were not considered. Also, studies of in vitro exposure to formaldehyde in solution and of exposure routes other than inhalation, which may inform mechanistic understanding, were initially kept for possible further review or qualitative

[17] Also identified from the NRC review and considered, but not ultimately included, in this section: (Qian et al., 1988) (an abstract); (Pongsavee, 2011) (ex vivo exposure to nongaseous formaldehyde; did not meet the inclusion criteria); and (Vargová et al., 1992) (evaluated and considered "not informative").

support of POE-related findings. However, given the large number of studies reporting results from inhalation exposure in vivo or gaseous exposure of airway cells, and considering the uncertainties associated with the toxicokinetics of noninhalation exposures, these comparably far less influential mechanistic data were ultimately not included in the final analysis described herein. These considerations informed the focus of the separate, systematic evidence map, developed to update the literature from 2017 to 2021 (see Appendix F).

Literature Search

A systematic evaluation of the literature database on studies examining potential mechanistic events pertaining to noncancer respiratory health effects in relation to formaldehyde exposure was initially conducted in August 2014, with yearly updates through 2017 (a separate Systematic Evidence Map updates the literature from 2017–2021 using parallel approaches, see Appendix F). The search strings used for the pre-2017 literature search were designed to emphasize identification of mechanistic effects related to inflammation or immune-related changes, as the expectation was that most other relevant mechanistic effects would be identified through the health effect-specific literature searches in Appendix Sections A.5.2–A.5.5. However, these strings (see Table A-62) returned a much wider range of studies than expected. Thus, the primary source of studies for this section comes from this specific literature search, while a small number of studies not identified through this search are included based on searches and screening protocols from the health effect-specific searches**.** Additional search strategies included:

- Addition of nonoverlapping (many references identified by the search terms in Table A-62 were also identified by health effect-specific literature searches) references describing mechanistic effects relevant to interpreting respiratory effects, as identified by other health effect-specific literature searches.

- Review of reference lists in the 2010 draft Toxicological Review for Formaldehyde (U.S. EPA, 2010), the ATSDR toxicological profile of formaldehyde (ATSDR, 1999), and the National Toxicology Program (NTP) report on carcinogens background document for formaldehyde (NTP, 2010). Note: although no specific references were added to the literature search as a result of this review, several references are footnoted as supportive information.

After manual review and removal of duplication citations, the articles identified from database searches were initially screened within an EndNote library for relevance; title and abstract were considered simultaneously in this process, followed by subsequent review of the full text. The search and screening strategy, including exclusion categories applied and the number of articles excluded within each exclusion category, is summarized in Figure A-30. Based on this process, 140 studies were identified and evaluated for consideration in the Toxicological Review. Given the size of the database of mechanistic studies available for review, some constraints were placed on the studies considered for inclusion. Studies that failed to include a comparison to quantified formaldehyde exposure (e.g., levels; duration) were excluded. As noninhalation studies

poorly replicate the distribution of inhaled formaldehyde, studies of noninhalation exposure and nongaseous in vitro exposure were set aside for possible use (note: these were ultimately not included in the final analysis because EPA concluded that a sufficient number of mechanistic studies employing inhalation exposure were identified). Similarly, a single thesis identified during the literature search was ultimately not included in the final analysis. Given the multitude of potentially relevant studies returned, and because this review focuses on mechanisms most likely to be relevant to respiratory tract effects in humans, nonmammalian models and tissue systems other than those that might be related to formaldehyde-induced respiratory effects (i.e., other than studies of the respiratory tract, or circulatory or immune-related effects) were excluded. The specific inclusion and exclusion criteria used in the screening step are described in Table A-63.

Table A-62. Summary of supplemental literature search terms for mechanistic studies relevant to potential noncancer respiratory health effects

Database	Search (no date limit thru 8/31/2014)
PubMed searched 9/4/2014	(*formaldehyde OR formalin) AND ("Adaptive immunity" OR asthma OR "atopic dermatitis" OR immune OR "innate immunity" OR redox OR allergic OR allergy OR "mucosal immunity" OR Eosinophil* OR Inflammation OR "Lung function test" OR "Nitric oxide" OR Wheezing OR rhinosinusitis OR lymphocyte OR bronchiolitis OR glucocorticoid OR IgE OR basophil OR "histamine-releasing factor" OR "mast cell" OR "reactive nitrogen species" OR "reactive oxygen species" OR "oxidative stress" OR isoprostane OR "Airway remodeling" OR phagocytosis OR "toll-like" OR "respiratory immunity" OR autoimmune OR interleukin OR "immune system" OR "allergic rhinitis" OR "chronic obstructive pulmonary disease" OR copd OR corticosteroids OR "Chronic bronchitis" OR fibrocyte OR hematopoie* OR "Epithelial injury" OR "epithelial repair" OR Th17 OR "Airway hyperresponsiveness" OR "Airway smooth muscle" OR "airway hyperreactivity" OR "Bronchoalveolar lavage" OR neutrophil OR cytokine OR Bronchiectasis OR th2 OR th9 OR "t cell" OR leukotriene OR "Bronchial epithelial cell" OR "Dendritic cell" OR Endothelin OR "growth factor" OR Lipoxins OR Prostaglandin OR cyclooxygenase OR "matrix metalloproteinase" OR ovalbumin OR "tumor necrosis factor" OR Phosphodiesterase OR "Bronchopulmonary dysplasia" OR Adipokine OR Eicosanoid OR bronchoconstriction OR Phospholipase OR Hyperpnoea OR bronchiectasis OR "corticosteroid responsiveness" OR "Type 2" OR "muscarinic receptor antagonism" OR "obstructive airway" OR Immunomodulation OR lipocalins OR allergen OR corticosteroids OR "Vascular endothelial growth factor" OR bronchiectasis OR immunodeficiency OR "Muscarinic receptor" OR *inflammatory OR Complement OR "Myeloid suppressor cell" OR immunoglobulin OR mucin OR Autophagy OR Leukocyte OR macrophage OR BALT OR "extracellular lining fluid") NOT (nocicept* OR pain OR "formalin test" OR "formalin-induced" OR "formaldehyde-fixed" or "formalin-fixed" OR "paraformaldehyde-fixed" OR "formaldehyde fixation" OR "formalin fixation" OR "10% formalin" OR "10% buffered formalin" OR "10% neutral buffered formalin" OR vaccin* OR inactivated OR "formalin-killed" or "formaldehyde-killed" OR dental OR formalinized)
Web of Science searched 9/5/2014	(TS=("formaldehyde" OR "formalin") AND TS=("Adaptive immunity" OR "asthma" OR "atopic dermatitis" OR "immune" OR "innate immunity" OR "redox" OR "allergic" OR "allergy" OR "mucosal immunity" OR Eosinophil* OR "Inflammation" OR "Lung function test" OR "Nitric oxide" OR "Wheezing" OR "rhinosinusitis" OR "lymphocyte" OR "bronchiolitis" OR "glucocorticoid" OR "IgE" OR "basophil" OR "histamine-releasing factor" OR "mast cell" OR "reactive nitrogen species" OR "reactive oxygen species" OR "oxidative stress" OR "isoprostane" OR "Airway remodeling" OR "phagocytosis" OR "toll-like" OR

Database	Search (no date limit thru 8/31/2014)
	"respiratory immunity" OR "autoimmune" OR "interleukin" OR "immune system" OR "allergic rhinitis" OR "chronic obstructive pulmonary disease" OR "copd" OR "corticosteroids" OR "Chronic bronchitis" OR "fibrocyte" OR hematopoie* OR "Epithelial injury" OR "epithelial repair" OR "Th17" OR "Airway hyperresponsiveness" OR "Airway smooth muscle" OR "airway hyperreactivity" OR "Bronchoalveolar lavage" OR "neutrophil" OR "cytokine" OR "Bronchiectasis" OR "th2" OR "th9" OR "t cell" OR "leukotriene" OR "Bronchial epithelial cell" OR "Dendritic cell" OR "Endothelin" OR "growth factor" OR "Lipoxins" OR "Prostaglandin" OR "cyclooxygenase" OR "matrix metalloproteinase" OR "ovalbumin" OR "tumor necrosis factor" OR "Phosphodiesterase" OR "Bronchopulmonary dysplasia" OR "Adipokine" OR "Eicosanoid" OR "bronchoconstriction" OR "Phospholipase" OR "Hyperpnoea" OR "bronchiectasis" OR "corticosteroid responsiveness" OR "Type 2" OR "muscarinic receptor antagonism" OR "obstructive airway" OR "Immunomodulation" OR "lipocalins" OR "allergen" OR "corticosteroids" OR "Vascular endothelial growth factor" OR "bronchiectasis" OR "immunodeficiency" OR "Muscarinic receptor" OR *inflammatory OR "Complement" OR "Myeloid suppressor cell" OR "immunoglobulin" OR "mucin" OR "Autophagy" OR "Leukocyte" OR "macrophage" OR "BALT" OR "extracellular lining fluid")) NOT TS=(nocicept* OR "pain" OR "formalin test" OR "formalin-induced" OR "formaldehyde-fixed" OR "formalin-fixed" OR "paraformaldehyde-fixed" OR "formaldehyde fixation" OR "formalin fixation" OR "10% formalin" OR "10% buffered formalin" OR "10% neutral buffered formalin" OR vaccin* OR "inactivated" OR "formalin-killed" or "formaldehyde-killed" OR "dental" OR "formalinized") Indexes=SCI-EXPANDED, CPCI-S, BKCI-S, BKCI-SSH Timespan=All years
Toxline searched 9/3/2014	**Part 1** @SYN0+@AND+(@OR+"Adaptive+immunity"+asthma+"atopic+dermatitis"+immune+"innate+immunity"+redox+allergic+allergy+"mucosal+immunity"+Eosinophil*+Inflammation+"Lung+function+test"+"Nitric+oxide"+Wheezing+rhinosinusitis+lymphocyte+bronchiolitis+glucocorticoid+IgE+basophil+"histamine-releasing+factor"+"mast+cell"+"reactive+nitrogen+species"+"oxidative+stress"+isoprostane+"Airway+remodeling"+phagocytosis+"toll-like"+"respiratory+immunity"+autoimmune+interleukin+"immune+system"+"allergic+rhinitis"+"chronic+obstructive+pulmonary+disease")+(@OR+formaldehyde+formalin+@term+@rn+50-00-0)+@NOT+(@OR+nocicept*+pain+"formalin+test"+"formalin-induced"+"formaldehyde-fixed"+"formalin-fixed"+"paraformaldehyde-fixed"+"formaldehyde+fixation"+"formalin+fixation"+"buffered+formalin"+"neutral+buffered+formalin"+vaccin*+inactivated+"formalin-killed"+"formaldehyde-killed"+dental+formalinized)+@NOT+@org+pubmed+pubdart+"NIH+reporter"
	@SYN0+@AND+(@OR+"Adaptive+immunity"+asthma+"atopic+dermatitis"+immune+"innate+immunity"+redox+allergic+allergy+"mucosal+immunity"+Eosinophil*+Inflammation+"Lung+function+test"+"Nitric+oxide"+Wheezing+rhinosinusitis+lymphocyte+bronchiolitis+glucocorticoid+IgE+basophil+"histamine-releasing+factor"+"mast+cell"+"reactive+nitrogen+species"+"oxidative+stress"+isoprostane+"Airway+remodeling"+phagocytosis+"toll-like"+"respiratory+immunity"+autoimmune+interleukin+"immune+system"+"allergic+rhinitis"+"chronic+obstructive+pulmonary+disease")+(@OR+formaldehyde+formalin+@term+@rn+50-00-0)+@NOT+(@OR+nocicept*+pain+"formalin+test"+"formalin-induced"+"formaldehyde-fixed"+"formalin-fixed"+"paraformaldehyde-fixed"+"formaldehyde+fixation"+"formalin+fixation"+"buffered+formalin"+"neutral+buffered+formalin"+vaccin*+inactivated+"formalin-killed"+"formaldehyde-killed"+dental+formalinized)+@AND+@org+"nih+reporter"

Database	Search (no date limit thru 8/31/2014)
	Part 2 @SYN0+@AND+(@OR+copd+corticosteroids+"Chronic+bronchitis"+fibrocyte+hematopoie*+"Epithelial+injury"+"epithelial+repair"+Th17+"Airway+hyperresponsiveness"+"Airway+smooth+muscle"+"airway+hyperreactivity"+"Bronchoalveolar+lavage"+neutrophil+cytokine+Bronchiectasis+th2+th9+"t+cell"+leukotriene+"Bronchial+epithelial+cell"+"Dendritic+cell"+Endothelin+"growth+factor"+Lipoxins+Prostaglandin+cyclooxygenase+"matrix+metalloproteinase"+ovalbumin+"tumor+necrosis+factor"+Phosphodiesterase+"Bronchopulmonary+dysplasia"+Adipokine+Eicosanoid+bronchoconstriction+Phospholipase+Hyperpnoea+bronchiectasis+"corticosteroid+responsiveness"+"Type+2"+"muscarinic+receptor+antagonism"+"obstructive+airway"+Immunomodulation+lipocalins+allergen+corticosteroids+"Vascular+endothelial+growth+factor"+bronchiectasis+immunodeficiency+"Muscarinic+receptor"+inflammatory+Complement+"Myeloid+suppressor+cell"+immunoglobulin+mucin+Autophagy+Leukocyte+macrophage+BALT+"extracellular+lining+fluid")+(@OR+formaldehyde+formalin+@term+@rn+50-00-0)+@NOT+(@OR+nocicept*+pain+"formalin+test"+"formalin-induced"+"formaldehyde-fixed"+"formalin-fixed"+"paraformaldehyde-fixed"+"formaldehyde+fixation"+"formalin+fixation"+"buffered+formalin"+"neutral+buffered+formalin"+vaccin*+inactivated+"formalin-killed"+"formaldehyde-killed"+dental+formalinized)+@NOT+@org+pubmed+pubdart+"NIH+reporter"
	@SYN0+@AND+(@OR+copd+corticosteroids+"Chronic+bronchitis"+fibrocyte+hematopoie*+"Epithelial+injury"+"epithelial+repair"+Th17+"Airway+hyperresponsiveness"+"Airway+smooth+muscle"+"airway+hyperreactivity"+"Bronchoalveolar+lavage"+neutrophil+cytokine+Bronchiectasis+th2+th9+"t+cell"+leukotriene+"Bronchial+epithelial+cell"+"Dendritic+cell"+Endothelin+"growth+factor"+Lipoxins+Prostaglandin+cyclooxygenase+"matrix+metalloproteinase"+ovalbumin+"tumor+necrosis+factor"+Phosphodiesterase+"Bronchopulmonary+dysplasia"+Adipokine+Eicosanoid+bronchoconstriction+Phospholipase+Hyperpnoea+bronchiectasis+"corticosteroid+responsiveness"+"Type+2"+"muscarinic+receptor+antagonism"+"obstructive+airway"+Immunomodulation+lipocalins+allergen+corticosteroids+"Vascular+endothelial+growth+factor"+bronchiectasis+immunodeficiency+"Muscarinic+receptor"+inflammatory+Complement+"Myeloid+suppressor+cell"+immunoglobulin+mucin+Autophagy+Leukocyte+macrophage+BALT+"extracellular+lining+fluid")+(@OR+formaldehyde+formalin+@term+@rn+50-00-0)+@NOT+(@OR+nocicept*+pain+"formalin+test"+"formalin-induced"+"formaldehyde-fixed"+"formalin-fixed"+"paraformaldehyde-fixed"+"formaldehyde+fixation"+"formalin+fixation"+"buffered+formalin"+"neutral+buffered+formalin"+vaccin*+inactivated+"formalin-killed"+"formaldehyde-killed"+dental+formalinized)+@AND+@org+"nih+reporter"

Abbreviations: Majr = major topic (filter); TS = the requested "topic" is included as a field tag.

1

Table A-63. Inclusion and exclusion criteria for mechanistic studies relevant to potential noncancer respiratory health effects

	Included	Excluded
Population	• Experimental animals • Humans	• Irrelevant species or matrix, including nonanimal species (e.g., bacteria) and studies of inorganic products
Exposure	• Quantified (e.g., levels; duration) exposure to formaldehyde in indoor air	• Not specific to formaldehyde (e.g., other chemicals) • No specific comparison to formaldehyde exposure alone (e.g., formaldehyde levels, duration, or similar in a study of exposure to a mixture)—NOTE: full text screening only • Nonrelevant exposure paradigm (e.g., use as a pain inducer in nociception studies) • Outdoor air exposure
Comparison	• Inclusion of a comparison group (e.g., pre- or postexposure; no exposure; lower formaldehyde exposure level)	• Case reports (selected references used for illustration)
Outcome	• Examining mechanistic endpoints relevant to interpretions of potential respiratory health effects	• Not relevant endpoints for section, including carcinogenicity studies and endpoints related to contact dermatitis • Exposure or dosimetry studies • Use of formaldehyde in methods (e.g., for fixation) • Processes related to endogenous formaldehyde • Related to hazard endpoints only (including genotoxicity; see those hazard sections)—NOTE: full text screening only
Other	• Original primary research article	• Not a unique, primary research article, including reviews, reports, commentaries, meeting abstracts, duplicates, or untranslated foreign language studies (these were determined to be off topic or unlikely to have a significant impact based on review of title, abstract, and/or figures).

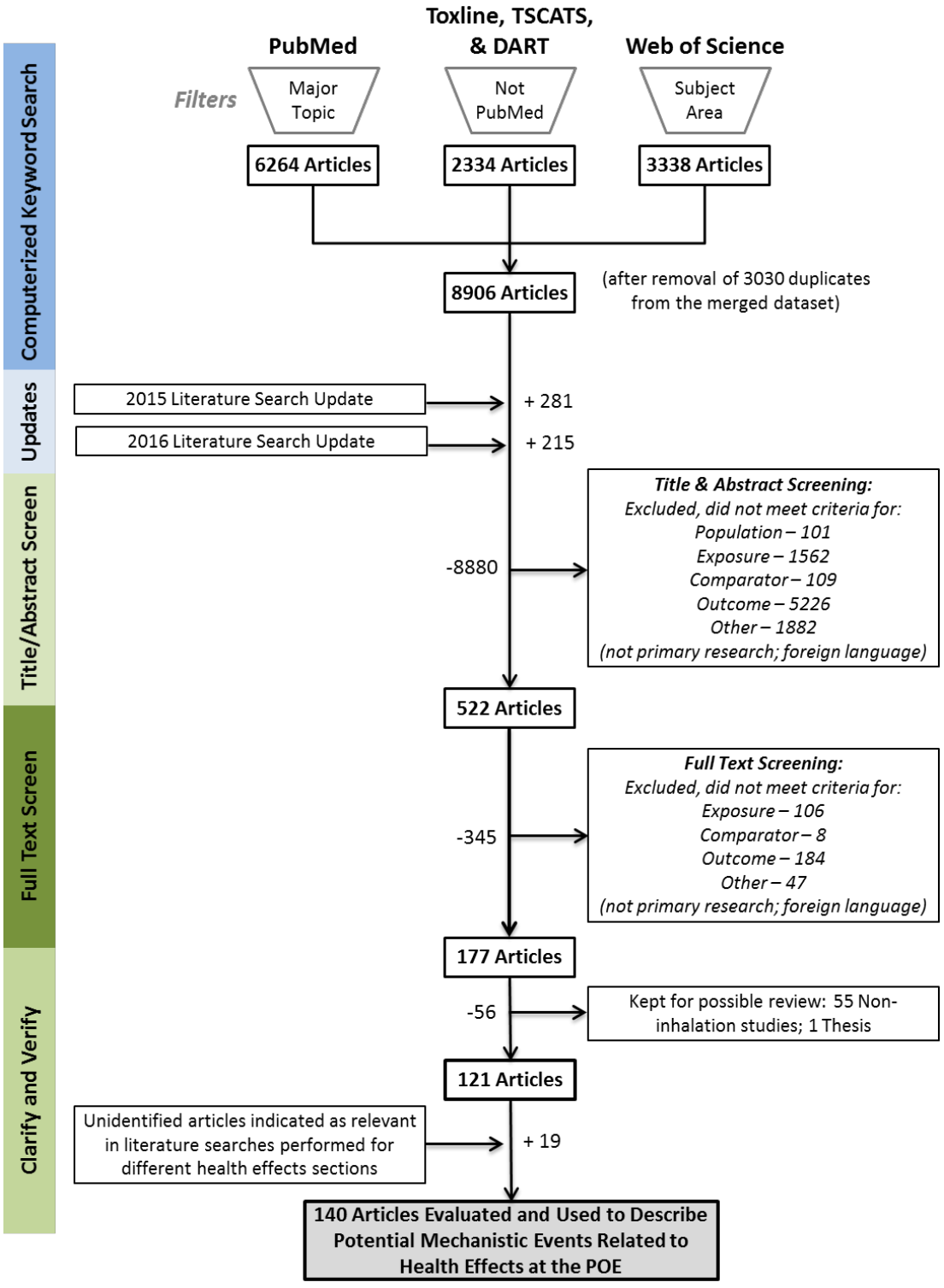


Figure A-30. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and mechanistic data associated with potential noncancer effects on the respiratory system (reflects studies identified in searches conducted through September 2016; see Appendix F for literature identification from 2016–2021).

Organizing and judging the evidence for mechanistic events and associations between events

Due to the importance of considering the toxicokinetics of inhaled formaldehyde, the human and animal experiments interpreted with *high* or *medium* confidence and *low* confidence were organized according to the tissue compartment and general type of change being examined. Individual experiments or groups of closely related experiments across studies were divided into mechanistic events, representing empirically observable biological changes that may inform how formaldehyde exposure might be associated with a respiratory health effect(s). *Mechanistic event* is used in this section as a generic term for types of endpoints, which may or may not be required for—or even influence—a mode of action; thus, mechanistic events are not necessarily *key events*, which are necessary precursor steps (or markers of such) in a mode of action (U.S. EPA, 2005). The level of evidentiary support for each mechanistic event was characterized based on the criteria presented in Table A-64. These criteria emphasize the confidence and consistency of the data across studies. Other relevant considerations (e.g., effect magnitude, dose-response, coherence) are discussed when conclusions across studies could be drawn, but these judgments were often difficult due to the heterogeneous nature of the available mechanistic studies. This section presents the broad conclusions drawn from sets of related studies.

Potential associations between mechanistic events were judged based on the tissue(s)/region(s) assessed and known biological roles within those tissues for the identified mechanistic events. The basis for each association was not individually documented, but these are generally discussed in the synthesis sections below and/or the study evaluation tables in the "Study Evaluations" section below.

Table A-64. Criteria and presentation of strength of the evidence for each mechanistic event and for potential associations between events relating to potential respiratory health effects

	Evidence judgment[a]	Mechanistic events		Associations between mechanistic events	
		Criteria for conclusions	***Presentation***	***Criteria for conclusions***	***Presentation***
STRONGEST	*Robust*	Direct evidence supporting an effect in multiple, consistent *high or medium confidence* studies [b]	Emphasized in Text	Formaldehyde-specific data demonstrate a linkage (i.e., inhibition of mechanistic event "A" prevents or reduces the occurrence of event "B"; events "A" and "B" are linked by concentration, location, and temporality)	→
	Moderate	Direct or indirect (e.g., genetic changes) evidence supporting an effect in at least 1 *high or medium confidence* study, with supporting evidence (e.g., consistent changes suggesting an effect in *low confidence* studies) [b]	Emphasized in Text	• An association between events "A" and "B" is known based on established (basic) biology • An association has been demonstrated for similar chemicals and/or effects	–→
	Slight	• Evidence supporting an effect in 1 hypothesis-generating *high or medium confidence* study • Evidence suggesting an effect in multiple, reasonably consistent *low confidence* studies	Minimal Discussion in Text	An association is justifiable, or even expected, based on underlying biology, but it has not been well-established (note: events for which an association is unlikely based on established understanding of underlying biology are not linked)	⇢
WEAKEST	*Indeterminate*	• Evidence suggesting an effect in 1 *low confidence* study • A set of *low confidence* studies with inconsistent results	Not included in figures; may be noted in text	N/A	N/A
		• Evidence cannot be interpreted (no data; no pattern in results within and/or across studies) • Data suggest no change	Not included in figures or synthesis text	N/A	N/A

[a]For consistency, the judgments used to describe the within-stream conclusions for apical health effect endpoints were applied, although the criteria used herein were less rigorous (i.e., when evaluating individual studies and sets of studies). Unlike within-stream conclusions, these terms are not bolded as they do not reflect evidence stream conclusions.

[b]The presence of a comparable or stronger set of studies with directly conflicting evidence results in the identification of the next weaker evidence descriptor (e.g., *robust* evidence with conflicting data would be *moderate*); note that the purpose of this evaluation was not to identify mechanistic events for which there was *robust* evidence of no change; however, the plausibility of the pathways (considering evidence for a lack of changes in expected events) is discussed in later sections.

Display and analysis of the mechanistic evidence

This chapter first describes the data for mechanistic events within each of the assessed tissue locations, and then analyzes the most informative data (i.e., preference is given to *robust* evidence) integrated across tissue compartments, both of which highlight potential effects on specific tissue components and/or functions. Both analyses include a discussion of the mechanistic events interpreted as the most likely to be due to (or most closely related to) direct interactions with inhaled formaldehyde molecules (i.e., "plausible initial effects of exposure"), as well as important apical toxicity endpoints (i.e., "key features of a potential hazard") discussed in previous sections (see Sections 1.2 and 1.3 in the Toxicological Review). In the first portion of this section, the network-based presentation serves to evaluate the interconnectivity of mechanistic changes within and across tissue compartments, and across potential noncancer respiratory system health effects. As an integrated overview, the analysis focuses primarily on the mechanistic events with *robust* and *moderate* evidence of formaldehyde-induced changes (see Figure A-31), but also includes consideration of the mechanistic events with *slight* evidentiary support (see Figure A-32). Where data clearly suggest a dependence on exposure duration or exposure level to elicit an effect, these associations are discussed. Note that this illustration is likely not a comprehensive picture of all potential formaldehyde-induced mechanistic changes or interactions between events, as it is based exclusively on events for which formaldehyde-specific data are available and which were captured by the literature search and screening process described above.

In the latter portion of this section, the network of mechanistic changes across tissues is distilled to the subsets of evidence that best link initial effects of formaldehyde inhalation in a linear fashion to key features for each of the noncancer respiratory system health effects evaluated in previous sections (see Figure A-34). In this analysis, for each of the more apical toxicity endpoints, the sequence of events interpreted to have the most reliable evidence (e.g., mechanistic events and associations with *robust* evidence are preferred) from a "plausible intial effect of exposure" are organized in a linear fashion, regardless of tissue region. This latter analysis attempts to simplify the data and emphasize the mechanistic events supported by the evidence interpreted with the highest confidence, but it is not intended to convey the majority of the available information. Aspects of this latter analysis are similar to components of the adverse outcome pathway (AOP) approach (Villeneuve et al., 2014; Ankley et al., 2010). These analyses only consider mechanistic events identified in formaldehyde-specific studies. The data supporting each sequence of events depicted in Figure A-32 are summarized into an interpretation regarding the biological plausibility of that sequence being a mechanism by which formaldehyde exposure might cause noncancer respiratory health effects. The synthesis text focuses on generalized summary findings regarding the identified mechanistic events rather than observations in individual studies. Thus, individual study references are not frequently cited in the text; these specific supporting references can be found in the tables at the end of each tissue compartment-specific section (see Tables A-66 to A-72).

Display and analysis of the mechanistic evidence

This chapter first describes the data for mechanistic events within each of the assessed tissue locations, and then analyzes the most informative data (i.e., preference is given to *robust* evidence) integrated across tissue compartments, both of which highlight potential effects on specific tissue components and/or functions. Both analyses include a discussion of the mechanistic events interpreted as the most likely to be due to (or most closely related to) direct interactions with inhaled formaldehyde molecules (i.e., "plausible initial effects of exposure"), as well as important apical toxicity endpoints (i.e., "key features of a potential hazard") discussed in previous sections (see Sections 1.2 and 1.3). In the first portion of this section, the network-based presentation serves to evaluate the interconnectivity of mechanistic changes within and across tissue compartments, and across potential noncancer respiratory system health effects. As an integrated overview, the analysis focuses primarily on the mechanistic events with *robust* and *moderate* evidence of formaldehyde-induced changes (see Figure A-31), but also includes consideration of the mechanistic events with *slight* evidentiary support (see Figure A-32). Where data clearly suggest a dependence on exposure duration or exposure level to elicit an effect, these associations are discussed. Note that this illustration is likely not a comprehensive picture of all potential formaldehyde-induced mechanistic changes or interactions between events, as it is based exclusively on events for which formaldehyde-specific data are available and which were captured by the literature search and screening process described above.

In the latter portion of this section, the network of mechanistic changes across tissues is distilled to the subsets of evidence that best link initial effects of formaldehyde inhalation in a linear fashion to key features for each of the noncancer respiratory system health effects evaluated in previous sections (see Figure A-34). In this analysis, for each of the more apical toxicity endpoints, the sequence of events interpreted to have the most reliable evidence (e.g., mechanistic events and associations with *robust* evidence are preferred) from a "plausible intial effect of exposure" are organized in a linear fashion, regardless of tissue region. This latter analysis attempts to simplify the data and emphasize the mechanistic events supported by the evidence interpreted with the highest confidence, but it is not intended to convey the majority of the available information. Aspects of this latter analysis are similar to components of the adverse outcome pathway (AOP) approach (Villeneuve et al., 2014; Ankley et al., 2010). These analyses only consider mechanistic events identified in formaldehyde-specific studies. The data supporting each sequence of events depicted in Figure A-34 are summarized into an interpretation regarding the biological plausibility of that sequence being a mechanism by which formaldehyde exposure might cause noncancer respiratory health effects. The synthesis text focuses on generalized summary findings regarding the identified mechanistic events rather than observations in individual studies. Thus, individual study references are not frequently cited in the text; these specific supporting references can be found in the tables at the end of each tissue compartment-specific section (see Tables A-66 to A-72).

Study Evaluations

Because a large number of relevant articles (mostly experimental studies with multiple, relevant endpoints) were considered in this analysis, a method was developed to distinguish the experiments likely to provide the most useful information from those providing less informative data or a comparably negligible amount of information. Individual mechanistic studies were evaluated using basic screening-level criteria (see Table A-65) for each relevant endpoint or group of related endpoints (e.g., hematological parameters) assessed by the study authors; thus, a study may be evaluated multiple times. Expert judgment of the totality of the potential limitations was used to determine a final level of confidence in the utility of the study results, with the reasoning documented. In some instances, notation is included regarding the sensitivity of the methods and whether they can provide information with direct relevance to interpreting cellular, structural, or functional changes related to potential respiratory system health effects. Although this information was not used in study evaluations, it was considered when developing the synthesis.

The study evaluation decision criteria were different for observational epidemiology studies and experimental studies, although both sets of criteria emphasized exposure-related considerations. As such, Tables A-66 to A-72 are first organized according to mechanistic effect type, and then within each effect type into observational and controlled exposure studies. The intent of the criteria applied, and the purpose of this mechanistic evaluation, was to focus on potential mechanisms associated with constant, chronic inhalation exposure to formaldehyde. Some studies of other effects that might be related to respiratory health effects have been evaluated in other sections of the Appendix and support evaluations of potential respiratory hazards; these evaluations informed the interpretation of overlapping studies presented in this section, as well as in the MOA analyses presented in the toxicological review. Studies of cellular proliferation, mucociliary function, and genotoxicity were separately reviewed, with the relevant conclusions directly incorporated into the MOA analyses described in the Toxicological Review. The application of the decision criteria presented in Table A-65 to the identified mechanistic studies is presented. Interpretations of the usefulness of the individual mechanistic studies for evaluating the effect(s) in question were drawn based on the results of applying the decision criteria. These interpretations were *high or medium confidence*—experiments considered very useful for describing potential formaldehyde inhalation-induced effects (since both medium and high confidence studies were considered well conducted, additional criteria were not applied to distinguish one from the other). In contrast, *low confidence* experiments might provide useful information, but should be considered in the context of other available data. *Not informative* studies were interpreted as providing negligible information regarding the potential for formaldehyde inhalation to cause the effect(s) of interest and were ultimately not included in the mechanistic analyses, given the identified limitations and the large number of available studies. Note that studies evaluating tissues interpreted as unlikely to be contributing to respiratory health effects (e.g., liver) are included in the Appendix Tables A-66 to A-72, but are not included in the MOA analyses presented in the

Toxicological Review or the systematic evidence map; the relative importance and ultimate decision to not include such information in the mechanistic analyses may change if the conclusion regarding their lack of relevance to respiratory health effects were to change with additional, future research.

Table A-65. Decision criteria for the evaluation of mechanistic studies relevant to potential noncancer respiratory effects

Observational studies preferences	Experimental studies (human or animal, controlled exposure) preferences
Generally, (not strictly scored) studies were considered *low* confidence if they had multiple (2) unmet preferences and *not informative* if the majority of preferences were not met:	**Generally, (not strictly scored) studies were considered *low* confidence if they had multiple (2–3) unmet preferences and *not informative* if the majority of preferences were not met:**
Exposure duration • duration ≥5 d (acute exposures noted) • daily exposures of several hours	*System* • in vivo with nose-only or whole-body inhalation exposure
Exposure levels • inhaled concentration accurately quantified in exposed group • use of an appropriate referent group • exposure contrast expected to allow for detection of differences across groups	*Test article* • explicit use of paraformaldehyde (PFA) or methanol-free preparations of formaldehyde; note: experiments of non-URT tissues/models (including lung) were automatically "low confidence" if this preference was not met)
Comparability • endpoint result comparisons can discern effects of formaldehyde exposure alone (e.g., controlling for co-exposures, blinding)	*Exposure paradigm* • duration of ≥5 d (acute exposures noted) • periodicity of ≥5 hrs/d and ≥5 d/wk (if ≥1 d)
Sample size • >10 persons/ group to (theoretically) reduce variability	*Exposure levels* • inhaled concentration was quantified (as ppm, mg/L or mg/m^3) • at least one tested exposure level of ≤3 mg/m^3 (Note: studies only testing above 10 mg/m^3 were considered "excessive")
Reporting • clear description of methods • detailed, quantitative reporting of results	*Comparability* • endpoint result comparisons can discern effects of formaldehyde exposure alone (e.g., controlling for other experimental manipulations, including chamber air exposure).
	Sample size • >10 humans or >5 animals/ group to (theoretically) reduce variability
	Reporting • clear description of methods • detailed, quantitative reporting of results

Evaluation of Individual Mechanistic Studies for Use in Describing Potential MOAs for Respiratory Effects

Important notes on Tables A-66 to A-72: Based on the assumption that most labs used commercially available formalin for convenience, the test article is assumed to be formalin (and is documented as such) if the test article was not reported; in some cases, multiple endpoints evaluated in the same row were interpreted as being informative to differing degrees; some specific, more apical endpoints described in the previous hazard sections are excluded from these tables; N/R= not reported; FA= formaldehyde). Studies on the implications of altered endogenous formaldehyde levels are not extracted into the tables below, although there may be some contextual discussion (e.g., to inform potential susceptibility) in the Toxicological Review.

Table A-66. URT-specific structural modification, sensory nerve-related changes, or immune and inflammation-related changes

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
Observational Epidemiology Studies					
(Lyapina et al., 2004)	Symptomatic and nonsymptomatic human workers with carbamide-FA glue (n=29)	Exposed workers: 0.8 7± 0.39 mg/m^3 (n=21 nonexposed); duration mean: 12.7 ± 9.6 yrs	Assessment of chronic URT inflammation	Statistically significant increase in subjective symptoms and objective clinical findings of chronic, URT inflammation (e.g., hypertrophy/atrophy of mucus membranes; rhinitis) and decreased neutrophil function (but N/C in leukocyte cell counts) in workers; symptomatic workers exhibited decreased resistance to infections (increased frequency, duration)	**High or Medium Confidence [mixture exposure]**
(Bono et al., 2016)	Human plastic laminate workers (n=50) and office personnel controls (n=45); males only	Controls (mean ± SE and range): 0.035 ± 0.0034 (0.016−0.11) mg/m^3; Workers: 0.211 ± 0.015 (0.049−0.444); duration unclear	Nasal epithelial ROS (M_1dG adducts; a marker of oxidative stress and lipid peroxidation)	Increased adducts with increasing formaldehyde exposure (p trend= 0.002), with statistically significant increases at > 0.066 mg/m^3 (i.e., <0.025 mg/m^3 = 47.6; 0.025−0.066 mg/m^3 = 59.2; and >0.066 mg/m^3 = 105.5 adducts)	**High or Medium Confidence [unknown duration]**
(Holmström and	Two exposed groups (n= 170 total; ≈90% male); 70	Exposed workers: chemical plant: 0.05−0.5 mg/m^3, mean 0.26 [SD 0.17 mg/m^3]. Furniture	Symptoms of URT inflammation Histopathology scores	Symptoms of nasal obstruction and nasal watery discharge more frequent in exposed (p <0.05). When divided into subgroups based on exposure	**Low Confidence [Inclusion of only current workers and long duration of employment raises possibility of healthy worker**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
Wilhelmsson, 1988) (note: mucociliary function data below)	formaldehyde production workers; 100 workers exposed to wood dust and formaldehyde at five furniture factories; Referent: (n=36; ≈55% male) from government, with no history of formaldehyde or wood dust exposure	factory: 0.2−0.3 mg/m^3, mean 0.25 [SD 0.05 mg/m^3]. Referent mean 0.09 mg/m^3 (based on 4 measurements in 4 seasons); duration of employment >10 yrs		time, there were no signs of increasing nasal restrictivity after employment >5 yrs. Formaldehyde-only nasal specimens mean histological score: 2.16 (range 0−4) (p <0.05) compared to referent group 1.56 (range 0−4); while formaldehyde-dust group had mean score 2.07 (range 0−6) (p >0.05). No correlation observed between smoking habits and biopsy score, nor was a correlation found between the duration of exposure and any histological changes.	**survival effect due to irritation effects; referent group not well matched (different type of work activity; undersampled males); crude measures of effect**
(Norback et al., 2000)	Primary school personnel in Sweden (n=234)	0.003−0.016 (mean= 0.0095) mg/m^3; duration unclear (working at least 20 hr/wk; assumed length months or more)	Assessment of acoustic rhinometry and factors in nasal lavage	Formaldehyde was significantly associated with multiple measures of nasal obstruction Formaldehyde was positively associated with biomarkers for eosinophils (eosinophil cationic protein; lysozyme); N/C in a neutrophil marker (myeloperoxidase) or albumin	**Low Confidence [mixture exposure (formaldehyde was independently associated with these changes, but so were NO_2 and Aspergillis)-did not evaluate confounding; some school measures below the limit of detection]**
(Priha et al., 2004)	Human MDF board workers (n=22) versus wood dust (n=23) and nonexposed (n=15)	0.19± 0.11 mg/m^3 (MDF board) versus 0.11 ± 0.08 mg/m^3 (note: VOCs 3-fold higher in MDF than wood); pre- and post-8-hr workshift	Nasal lavage cell and cytokine counts	N/C in cell counts Increased postshift total protein vs. unexposed controls Increased post- vs. preshift NO (nitrite) in wood and MDF workers Decreased post- vs. preshift TNFα in wood workers	**Low Confidence [short duration; minimal exposure differential; role of VOCs not accounted for]** NOTE: ACUTE (8 hr; cross-shift)
Controlled-Exposure Studies in Humans or Primary Human Cells					
(Pazdrak et al., 1993)	Human occupationally exposed (n=10 males and	Formalin (assumed: test article NR): 0.5 mg/m^3 for 2 hr with follow-up out to 16−18hr	Nasal lavage cell and protein counts Note: changes were associated with	Increased number of eosinophils, albumin, and total protein; N/C basophils	**Low Confidence [formalin; short duration; somewhat small sample size; lack of investigator**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
	females) with positive reaction to FA: "allergic"; 11 "nonallergic" control males		scoring measures of nasal symptoms (e.g., sneezing; edema)	Increased proportion of eosinophils and decreased proportion of epithelial cells; N/C in proportion of basophils, neutrophils, or mononuclear cells (i.e., lymphocytes and monocytes) Effects max 10 min after exposure and declining, but still significant, at 16–18 hr; effects observed regardless of "allergy"	**blinding (nonissue for automated albumin measures)]** NOTE: ACUTE; authors noted albumin changes may indicate increased mucosal permeability: albumin percentage, also called the "permeability index," was elevated at 10 min postexposure only
(Krakowiak et al., 1998)	Human workers with bronchial asthma or healthy subjects (n=10 each)	Formalin (assumed: test article NR): 0.5 mg/m^3 for 2 hr with follow-up out to 24 hr	Nasal lavage cell and protein counts Note: changes were associated with scoring measures of nasal symptoms (e.g., sneezing; edema)	Increased eosinophils, leukocytes, total cell counts, and permeability index at 30 min after exposure, but not at 4 hr or 24hr after exposure; N/C in basophils (changes were observed regardless of asthmatic designation) N/C in mast cell tryptase or eosinophil cationic protein	**Low Confidence [formalin; short duration; small sample size; lack of investigator blinding (nonissue for automated albumin measures)]** NOTE: ACUTE; albumin percentage, aka "permeability index" was used to indicate mucosal permeability; no effect on FEV_1, etc.
(Falk et al., 1994)	Human sympto-matic for nasal distress (n=7) or controls (n=6)	Formalin (assumed from description of test article) Symptomatic: 0.021, 0.028, 0.073, 0.174 mg/m^3; ≤2 hr Healthy: 0.023, 0.29, 0.067, 0.127 mg/m^3; ≤2 hr	Nasal mucosa swelling by rhinostereometry	FA increased mucosal swelling at ≥0.073 mg/m^3 in symptomatic persons, but swelling was unchanged in healthy controls	**Low Confidence [formalin; short duration; small sample size]** NOTE: ACUTE; assay is relevant to inflammation, but limited in scope and exposure contrast
(He et al., 2005)	Human student volunteers (n=10)	Ocular exposure to wood-panel generated formaldehyde gas 0, 1, 2, or 3 mg/m^3; 5 min/d for 4 d	Nasal lavage substance P	Substance P was increased significantly at 3 mg/m^3	**Low Confidence [exposure route- unknown relevance of ocular exposure route to inhaled exposure level, but considered to be reasonable due to similarities in access of gas to trigeminal nerve endings for this endpoint; short**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
					duration and periodicity; somewhat small sample size]
(Bardet et al., 2014)	In vitro (human primary nasal cells); n=5 experiments (cells: one donor)	Formalin gas: 0.2 mg/m^3 for 1 hr/d for 1, 2, or 3 d	Nasal cell cytokine secretion (at 72 hrs for all exposures)	Slight, statistically significant, decreased IL-8 with 3 exposures only; N/C in IL-6	**Not Informative [in vitro; formalin; short duration; small sample size; comparable in vivo inhaled exposure level unknown]**
Controlled-Exposure Studies in Animals, Animal Cells, or Immortalized Human Cells					
(Fujimaki et al., 2004b)	Female C3H mice (n=5−6 per group)	PFA 0, 0.098, 0.49, or 2.46 mg/m^3; 12 wks	Serum cytokines and neuropeptides (see explanation at right)	D/D increased Substance P without OVA (no change + OVA) at 2.46 mg/m^3; FA decreased OVA-induced NGF elevation at 0.098−0.49 mg/m^3 (N/C with FA alone) Body weight decreased at ≥0.49 mg/m^3	**High or Medium Confidence [small sample size]** Note: although serum measure, discussed in the context of changes in the URT, so included here
		Sensitization: i.p. 10ug OVA prior to FA exposure; aerosol OVA boost for 6 min on wks 3, 6, 9, and 11			
(Monticello et al., 1989)	Young adult male rhesus monkeys (n=3/group)	PFA 0 or 7.38 mg/m^3 for 1 or 6 wk (6 hr/d, 5 d/wk)	Nasal histopathology	Goblet cell loss, hyperplasia and neutrophil inflammatory response at 1 wk	**High or Medium Confidence [high exposure level]** Note: n=3 monkeys/group considered a reasonable sample
(Andersen et al., 2010)	Male F344/CrlBR rats (n=7−8)	PFA 0, 0.86, 2.46, 7.38, 12.3, or 18.5 mg/m^3 for 1, 4, or 13 wk (6 hr/d, 5 d/wk)	Nasal histology Nasal mRNA analyses (Note: modeling results not considered)	mRNA changes: altered cellular immune response at 1 wk at 12.3−18.5 mg/m^3, with changes in DNA repair and cell cycle at ≥ 2.46 mg/m^3; by 4 wk, immune/injury response is lost; by 13 wk, pervasive changes noted	**High or Medium Confidence** Note: unclear, indirect interpretability of mRNA profiling
(Andersen et al., 2008)	Male F344 rats (n=8 for histopath; n ≥5 for genomics)	PFA 0, 0.86, 2.46, or 7.38 mg/m^3 for up to 3 wks (6 hr/d, 5 d/wk); also acute (18.5 mg/m^3) and instillation	Nasal histopathology, and microarray (high flux regions)	Inflammatory cell infiltration was observed at 7.38 mg/m^3 at ≥1-d exposure; microarray changes at ≥2.46 mg/m^3 at 5 d, but only at 7.38 mg/m^3 at 15 d (1 gene at 2.46 mg/m^3, 1 d); mostly stress-response related	**High or Medium Confidence** NOTE: unclear, indirect interpretability of genomic endpoints; note: nasal instillation caused more robust changes
(Woutersen et al., 1989)	Male Wistar rats (n>20/ group)	PFA 0, 0.12, 1.23, or 12.3 mg/m^3 for 28 mos (6 hr/d, 5 d/wk)	Nasal pathology	No treatment-related changes at 0.12−1.23 mg/m^3; evidence of damage, inflammation, proliferation at 12.3 mg/m^3	**High or Medium Confidence**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Rager et al., 2014)	Male Fischer rats (n=3 biological replicates/group)	PFA 0 or 2.46 mg/m^3 for 7 d, 28 d or 28 d with 7 d recovery (6 hr/d)	miRNA microarray of nasal respiratory epithelium	Nasal miRNAs were changed after 7 d or 28 d (84 or 59 transcripts), not with recovery; associated with inflammation and immunity, or tumor suppression	**High or Medium Confidence [very small sample size]** NOTE: unclear, indirect interpretability of endpoints
(Tsubone and Kawata, 1991)	Male Wistar rats (n=6/ group; each rat received 2−4 exposures of PFA or control air)	PFA 0.39-5.78 mg/m^3 through upper airway for 22 sec (under anesthesia)	Ethmoidal nerve activity (nasal trigeminal nerve branch)	Afferent nerve activity was increased by FA, with a 50% increase in activity at ≈2.2 mg/m^3 (although FA stimulated nerve activity at all levels- ≈20% at 0.62 mg/m^3)	**High or Medium Confidence [short duration]** NOTE: ACUTE: surgical procedures considered internally controlled (since rats served as own controls)
(Kulle and Cooper, 1975)	Male SD rats (n=5)	PFA 0.62, 1.23, 1.85, or 2.46 mg/m^3 for 1 hr or 0.62−3.08 mg/m^3 for 25 sec (with anesthesia)	Nasopalantine nerve responses (similar to ethmoidal in preliminary tests)	Sensory threshold from 25 sec exposure: 0.31 mg/m^3 Trigeminal response to an odorant (amyl alcohol) is decreased at ≥0.62 mg/m^3 FA	**High or Medium Confidence [slightly small sample size; short duration]** NOTE: ACUTE; surgical procedures internally controlled
(Yonemitsu et al., 2013)	TRPA1 knockout (KO) or wild type (WT) mice (n=3−5)	Formalin at up to 123 mg/m^3 (varied by experiment and chamber location, but all exposures considered "excessive"); ACUTE	Responses related to effects on the trigeminal nerve	Formalin vapor (3 min) activated secondary trigeminal system neurons (according to c-fos activity) in WT but not KO mice. Consistent with this, formalin vapor accelerated wakefulness and induced avoidance behaviors in WT but not KO mice; and labeling studies confirmed TRPA1 expression on trigeminal afferents innervating the nasal mucosa	**High or Medium Confidence [small sample size; short duration; formalin; excessive levels; see below for explanation]** NOTE: ACUTE; effects of related chemicals such as acrolein were similarly blocked in KO mice. Given the difficult nature of studying this event, the consistency of effects across related chemicals, and the well-accepted role for TRPA1 in acrolein-induced sensory effects (based largely on Bautista et al. (2006)), these results are judged to provide indirect evidence interpreted with high or medium confidence and not

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
					direct evidence interpreted with low confidence.
(Rager et al., 2013)	Male cynomolgus macques (n=2–3/group)	PFA 0, 2.46, or 7.38 mg/m^3 for 2 d (6 hr/d)	Nasal miRNA screen and molecular target verification	3 and 13 miRNAs were dysregulated by exposure, including associations with decreased apoptosis signaling (at 2) and increased epithelial proliferation (at 6)	**Low Confidence [short duration; n=2 primates: small sample size]** NOTE: Unclear direct relevance of miRNA changes
(Clement et al., 1987)	Female Wistar Rats (n=10)	PFA 0 or 18.5 mg/m^3 for 12 wks (6 hr/d, 5 d/wk)	URT epithelial structure and junctional proteins by IHC and TEM	Basal lamina degeneration, and goblet cell hypertrophy of respiratory epithelium FA reduced levels of junctional proteins but did not cause destroy the junctional complex when assessed by TEM Note: body weight significantly decreased by FA (<5%)	**Low Confidence [excessive exposure levels]**
(Cassee et al., 1996b)	Male Wistar albino rats (≥3/group)	PFA 0, 1.23, 3.94, or 7.87 mg/m^3 for 1 or 3 d (6 hr/d)	Nasal histopathology and biochemistry	Evidence of damage and inflammation at 3 d, ≥3.94 mg/m^3 Increased GPx and NPSH (3 d, ≥3.94 mg/m^3; latter at 1 d, 7.87 mg/m^3 too), not GST, FDH, ADH, or GR in respiratory epithelium	**Low Confidence [short duration; very small sample size]** NOTE: ACUTE or 3 d; NPSH: nonprotein sulfhydryl groups
(Cassee and Feron, 1994)	Male Wistar rats (n=20/ group; n=6+/endpoint)	PFA 4.43 mg/m^3 for 3 days (intermittent) Note: weights decreased in all groups	Nasal enzyme activity Nasal GSH	Increased GPx N/C in ADH, GST, G6PDH, GR, or FDH N/C in cytosolic GSH (slightly increased) Note: rhinitis and necrosis also reported	**Low Confidence [short duration and unclear periodicity; high exposure level]**
(Abreu et al., 2016)	C57BL/6 mice (n=12 M+F/ treatment group and n=6 M+F/control)	Formalin (assumed) 0, 0.25, 1.2, and 3.7 mg/m^3 for 8 hr (aldehyde mixture data not included herein; authors noted some exposure cross-contamination)	Nasal epithelial histology (morphology only) (blinded measures 6–8 hr postexposure)	N/C in nasal epithelium, except small, but significant, decreases in cilia at 0.25 mg/m^3	**Low Confidence [formalin; short duration and periodicity; some coexposure to acetaldehyde possible but unclear]** Note: ACUTE

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Monteiro-Riviere and Popp, 1986)	Male F344 rats (n=3 examined in detail)	PFA 0, 0.62, 2.46, 7.38, or 18.5 mg/m^3 for up to 4 d (6 hr/d); controls not air-exposed	URT respiratory epithelium ultra-structural pathology	Inflammation (neutrophil infiltration; goblet cell hypertrophy) at ≥7.38 mg/m^3; duration-dependency shown	**Low Confidence [short duration; very small sample size; controls not air exposed]** NOTE: no statistical comparisons of structural changes
(Mcnamara et al., 2007)	In vitro mouse and rat dorsal DRG neurons (n=300+ neurons) or HEK293 cells (n ≥ 5); (note: relevance is as URT stimulus)	Formalin or methanol controls (levels irrelevant to inhalation exposure); ACUTE experiments	Activation and specific inhibition of "sensory nerve cell" activity	Formalin, but not methanol, specifically activated TRPA1 in vitro. This specific activation was confirmed using TRPA1 knockout DRG neurons as well as specific pharmacologic inhibitors. TRPA1 inhibition also reduced formalin-induced pain behaviors in vivo.	**Low Confidence [in vitro; unknown exposure level relevance; short duration]** Note: ACUTE; methanol controls; categorized as low confidence rather than excluding due to less concern for methanol effects on receptors in nasal mucosa
(Tani et al., 1986)	Male rabbits (strain unspecified) n= unclear	Formalin 12.3 mg/m^3 (acute) directly infused into either the URT (nasal) and/ or LRT (lung)	Pharmacologic intervention studies on respiratory and cardiac function (compared to acrolein and ammonia)	The effects of formaldehyde on respiration and heart rate were only observed with nasal exposure, not lung. Inhibition of afferent sensory nerve activity abrogated the formaldehyde effects.	**Low Confidence [formalin; short duration; unknown sample size]** NOTE: ACUTE; categorized as low confidence rather than excluding due to less concern for methanol effects on receptors in nasal mucosa
(Kunkler et al., 2011)	In vitro trigeminal root ganglia (rat) neurons (n=9−15)	Formalin (levels irrelevant to inhalation exposure); ACUTE experiments	Agonist/antagonist studies of TRP channel-mediated CGRP release	Formaldehyde stimulated release of CGRP from adult trigeminal neurons (Note: inhibitor studies not tested on FA, but acrolein was through TRPA1)	**Low Confidence [in vitro; formalin; short duration; high, unknown exposure level]** NOTE: ACUTE; categorized as low confidence rather than excluding due to less concern for methanol effects on receptors in nasal mucosa
(Zhao et al., 2020)	Male Balb/c mice (n=3, pooled into single sample for nose and lung samples); 2 experiments	Formalin 0, 3 mg/m^3 for 2 wks (8 hr/d, 5 d/wk)	Burst-forming unit-erythroid (BFU-E), and colony-forming unit-granulocyte macrophage (CFU-GM) colonies in	Nose (ex vivo) results: Decreased formation of BFU-E in both experiment I and II Decreased formation of CFU-GM in experiment I; N/C in experiment II Nose (in vitro treatment):	**Low Confidence [formalin; small sample size; in vitro (for cell treatments)]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
	by different researchers		nose, lung, spleen, and bone marrow	400 uM formaldehyde significantly decreased BFU-E not CFU-GM formation (both nonsignificantly decreased across doses)	
(Hester et al., 2003)	Male F344 rats; n=3−4	Formalin (assumed, based on description); nasal instillation (400 mM in 4 0µL aliquot/nostril)	Respiratory epithelium gene expression	24 of 1,185 genes upregulated, and 22 downregulated	**Not Informative [formalin; short duration; very small sample size; high, unknown exposure level; exposure route]** NOTE: ACUTE
(Ohtsuka et al., 2003)	Male BN and F344 rats; n=4/group	Formalin aerosol 1% for 3 hr/d for 5 d vs. water	Nasal mucosa cytokines and structure	Degeneration and neutrophil inflammation (F344> BN) Decreased IFN-γ and IL-2 in BN; N/C in F344; N/C in IL-4 or IL-5 in BN or F344	**Not Informative [formalin; short periodicity; small sample size; high, unknown exposure levels]**
(Macpherson et al., 2007)	In vitro; n ≥ 7; transfected cells (HEK293T cells neuroendocrine; immortalized human kidney)	Formalin (levels irrelevant to inhalation exposure); ACUTE experiments	Activation and specific inhibition of "sensory nerve cell" activity	Formalin activated TRPA1. This selective activation was confirmed by inhibition of pain-related behaviors induced by formalin in vivo.	**Not Informative [in vitro; formalin; short duration; high, unknown exposure level; limited reporting]** NOTE: ACUTE

Table A-67. LRT (e.g., lung, trachea, BAL) markers of structural modification, immune response, inflammation, or oxidative stress

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
Observational Epidemiology Studies					
(Franklin et al., 2000)	Human healthy children (n= 224; age ≈9.5 yr);	FA levels in bedroom and living room were dichotomized into > or < 0.062 mg/m^3; duration unknown	exhaled nitric oxide (eNO); Note: technique used excludes NO originating from the upper airway	eNO ("reflects airway inflammation") significantly increased in children of homes with higher FA levels, after correcting for multiple other variables	**High or Medium Confidence [limited exposure contrast; accuracy of single measure questionable]** Note: authors suggest species differences in inflammation locale
(Bentayeb et al., 2015)	Human elderly (>65 yrs) European nursing home individuals (n=600 from 20 homes)	Indoor FA levels in main common room ranged from approximately 0.005–0.01 mg/m^3 (median ≈0.006) over 1 wk of sampling; duration unknown	eNO (marker of lower airway inflammation) eCO (marker of CO exhalation and smoking)	FA was not associated with eNO FA was associated with increased eCO Note: FA was associated with increased reported COPD and FVC, but not FEV1, asthma diagnosis or symptoms, or cough	**High or Medium Confidence [limited exposure contrast; unclear whether adjusted for co-exposures]** Note: PM co-exposure was not associated with eNO or eCO; NO_2 was associated with decreased eNO
(Flamant-Hulin et al., 2010)	Human school children (34 asthmatics; 70 nonasthmatics);	[Low] yards: 0.0036 (0.0024–0.0044) mg/m^3 and rooms: 0.025 (0.013–0.036) mg/m^3 [High] yards: 0.0058 (0.0049–0.0068) mg/m^3 and rooms: 0.044 (0.038–0.047) mg/m^3; unknown duration	Fractional exhaled nitric oxide (FeNO)—"reliable, noninvasive marker of airway inflammation" [Note: "nasal contamination" was prevented]	FeNO significantly increased in both nonasthmatics and asthmatics with high versus low FA exposure in classrooms, but not schoolyards; in nonasthmatics, a stronger association was found for atopic versus nonatopic children	**High or Medium Confidence [accuracy of single measure questionable]** Note: authors hypothesized that atopic status might modify airway response to formaldehyde; called changes "bronchial inflammation"
(Roda et al., 2011)	French infants (n=2,940 with assessment at birth and 12 mos)	Median 0.020 mg/m^3; IQR 0.014–0.027 mg/m^3; LOD 0.008 mg/m^3.	LRT infections (with or without wheeze) Note: although URT infections were queried, these data were NR	Significantly increased LRT infection: 32% or 41% increase per 0.0124 mg/m^3 increase in formaldehyde (without and with wheeze, respectively)	**High or Medium Confidence [specificity and sensitivity of predictive model not tested on a separate sample]**
(Rumchev et al., 2002)	Australian children (ages 6-	Mean 0.030 and 0.028 and maximum 0.224 and	Lower respiratory tract infection	Increased emergency room visits for this case definition	**Low Confidence [recruitment process not described;**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
	36 mos); 88 cases, 104 controls	0.190 mg/m^3, respectively, in bedroom and living room.	involving wheezing (assuming misclassification of a many of the discharges as asthma rather than infection)		**uncertainty as to how well this case definition describes LRT infection and the length of time between emergency room visit and subsequent exposure measure]**
Controlled-Exposure Studies in Humans or Primary Human Cells					
(Casset et al., 2006)	Human (n=19 with mild asthma and allergy to mite allergen)	Formalin 0.1 mg/m^3 for 30 min; placebo at ≈0.03 mg/m^3 double-blind randomized; restricted to mouth breathing only	Sputum (lower airway mucus) eosinophils and ECP	Authors note a trend, not statistically significant, towards increased eosinophil counts (≈38 ± 9% vs. 11 ± 3%, FA vs. air controls), and an increase in ECP (439 ± 171 vs. 156 ± 58 µg/l, FA vs. air controls)	**Low Confidence [formalin; short duration; not clear that restriction to mouth breathing is realistic for typical inhalation]** NOTE: ACUTE; within-subjects comparison between air and FA
(Ezratty et al., 2007)	Human (n=12 intermittent asthmatics with allergy to pollen)	Formalin 0.5 mg/m^3 for 60 min; randomized allocation (no nonexposed controls)	Sputum (lower airway mucus) cell counts and released factors	N/C in sputum Total cell counts, WBC subtypes, or factors (e.g., ILs, MCP, TNF)	**Low Confidence [formalin; short duration]** NOTE: all exposed to both air and FA; internally controlled
Controlled-Exposure Studies in Animals, Animal Cells, or Immortalized Human Cells					
(Fujimaki et al., 2004b)	Female C3H mice (n=5–6 per group)	PFA 0, 0.098, 0.49, 2.46 mg/m^3; 12 wks	BAL cell counts BAL cytokines and neuropeptides	No significant changes in cell counts with FA alone; macrophages and eosinophils increased at 2.46 mg/m^3 with OVA+FA; N/C in neutrophils or lymphocytes No significant changes in cytokines with FA alone (NGF was D/D increased) FA with OVA D/D decreased IL-1β at 2.46 mg/m^3 and NGF at 0.098–0.49 mg/m^3; N/C in TNF-α, GM-CSF, or IL-6; MCP-1, MIP-1a, and eotaxin were not detectable Body weight decreased at ≥0.49 mg/m^3	**High or Medium Confidence [small sample size for some groups/endpoints]** Note: MIP-1α, eotaxin, MCP-1, BDNF, and Substance P levels insufficient for testing
		Sensitization: i.p. 10 µg OVA prior to FA exposure; aerosol OVA boost for 6 min on wks 3, 6, 9, and 11			
		Formaldehyde (bottled pressurized gas) 0, 0.13,	Airway histology and morphometry	With FA, lung bronchi had intramural edema (wall thickening) by	**High or Medium Confidence [small sample size]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Riedel et al., 1996)	Female Dunkin-Hartley guinea pigs (n=3)	0.31 mg/m^3 for 5 d (8 hr/d) Sensitization: 0.5% inhaled OVA; OVA boost at 2wk Challenge: 1% inhaled OVA 1 wk later		morphometry; no evidence of cellular lower airway inflammation by histology	Note: histology after FA with OVA not examined
(Ito et al., 1996)	Male Wistar rats (n=7)	Formalin (with MeOH controls) 2.46, 6.15, 18.5, or 55.4 mg/m^3 for 10 min	Airway microvascular leakage (Evans blue) in trachea and main bronchi	D/D increased leakage at ≥6.15 mg/m^3, which resolved in <20 min Leakage at 18.5 mg/m^3 was inhibited by NK1 receptor antagonism, but not by hista-mine H1 or bradykinin B2 R antagonists 55.4 mg/m^3 MeOH alone induced slight leakage in main bronchi, but not trachea)	**High or Medium Confidence [short duration]** Note: figure comparisons presented against room air, not MeOH, controls, but comparisons made to MeOH controls in text
(Jakab, 1992)	In vivo and Ex vivo Female Swiss mice (n=5+ mice/ determination)	PFA 0,0.62 1.23, 6.15, 12.3, or 18.5 mg/m^3 for 4−18 hr or 4 d (4 hr/d); ± carbon black	Pulmonary bactericidal activity to inhaled Staphylococcus And ex vivo alveolar macrophage function	Pulmonary antibacterial activity was reduced: at 1.23 mg/m^3 for 18 hr before and 4 hr postbacterial challenge (postexposure alone reduced at 18.5 mg/m^3) N/C in ex vivo alveolar macrophage Fc receptor-mediated phagocytosis of RBCs at 6.15 mg/m^3 for 4 d (FA + carbon black, but not FA alone, caused a robust decrease)	**High or Medium Confidence [short duration]—in vivo pulmonary bactericidal activity** Note: ACUTE **Low Confidence [ex vivo; short duration]**
(Swiecichowski et al., 1993)	Male Hartley guinea pigs (n=5−12/group)	PFA at 4.18 mg/m^3 for 2 or 8 hrs (multiple experiments)	Airway Histology (trachea)	No change histological evidence of cell infiltration or epithelial damage up to 96 hr after exposure to 4.18 mg/m^3 for 8 hr	**High or Medium Confidence at 1.23 mg/m^3 and above [short duration]** **Low Confidence below 1.23 mg/m^3 and ex vivo [ex vivo; sample size of 5 at 1 or more levels below 1 ppm]** NOTE: ACUTE
(Ozen et al., 2003a)	Male albino Wistar rats (n=6)	PFA at 6.15 and 12.3 mg/m^3 for 4 or 13 wks (8 hr/d)	Lung tissue homogenate measures of trace elements	Zn was dose-dependently decreased (≥6.15 mg/m^3 for both exposure durations	**High or Medium Confidence [high levels]** NOTE: unclear relevance of endpoints; authors claim Fe

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
				Fe was dose-dependently increased (≥6.15 mg/m^3 with 13 wk; significant only at 12.3 mg/m^3 after 4 wk); Cu was unchanged	change linked to oxidative stress and Zn change linked to decreased DNA synthesis, but no direct evidence
(Aydin et al., 2014)	Male SD rats (n=6/group)	Test article unclear, but appears to be formalin in this experiment at 0, 6.48 (low), 12.3 (moderate), or 18.7 mg/m^3 for 4 wk (8 hr/d, 5 d/wk)	Lung tissue total antioxidant and total oxidant levels (TAS and TOS; kit uses vitamin E and H_2O_2 as reference, respectively Lung tissue oxidative stress index (OSI: TOS/TAS) and apoptotic index Lung irisin (hormone may regulate obesity)	Increased TOS and OSI, and decreased TAS and irisin, at ≥ 12.3 mg/m^3 formaldehyde Increased lung apoptotic index at ≥6.48 mg/m^3 Note: Carnosine supplementation reduced changes.	**Low Confidence [formalin; high levels]**
(Luo et al., 2013)	In vitro and ex vivo (intact trachea) from SD rats (sex NR); n= as low as 4 (some inhibitor assays), as high as 28 (trachea)	Formalin (assumed, test article NR; levels irrelevant to inhalation exposure); ACUTE (bath application) experiments	/sc currents in trachea and epithelium from trachea with various inhibitors TRPV channel expression and labeling	Formaldehyde caused a dose-dependent, sustained increase in currents in isolated trachea and airway epithelia TRPV-1 channels were localized to intraepithelial nerve endings and inhibition of TRPV-1 or substance P activity (blocking NK-1R) inhibited current increases Cl- released in response to formaldehyde was blocked several Cl channel blockers and involed cAMP	**Low Confidence [in vitro and ex vivo (intact trachea); formalin; unknown exposure level relevance]** Note: ACUTE, some inhibition experiments had n=4, but magnitude of inhibition was robust with small variabilty
(Lundberg and Saria, 1983)	Male SD rats (sample size NR)	Direct injection of formaldehyde (assumed to be formalin); 50 µL volume unknown comparison to inhalation exposure	Tracheal mucosal reactivity (Evans blue extravasation)	Formaldehyde injection caused extravasation which was reduced or abolished by capsaicin pretreatment	**Low Confidence [formalin; inferred high levels; short duration; nonspecific reporting]** NOTE: ACUTE

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Larsen et al., 2013)	Male BALB/cA mice (n=10/ group)	PFA 0.49, 2.21, or 4.9–7.0 (dry vs. humid air) mg/m^3; 60 min	BAL counts	FA did not affect BAL "degree of lung inflammation" (data not shown; unclear if this reflects comparisons of total cell counts or comparisons of individual cell types, as data were presented for OVA, i.e., neutrophils, lymphocytes, eosinophils, macrophages)	**Low Confidence [short duration; for BAL endpoints: poor reporting: FA alone groups data NR; OVA without FA and OVA with FA groups combined]** NOTE: ACUTE
		Sensitization: pre-FA i.p. 1 µg OVA, with 0.1 µg OVA boosts i.p. on days 14 and 21 (note: FA on day 31) Challenge: 0.2% OVA aerosol for 20 min on Days 29 and 30			
(Wu et al., 2013)	Male Balb/c mice (n=8/group)	Formalin 0 or 3 mg/m^3 for 4 wk (6 hr/d, 5 d/wk) with or without OVA aerosol	BALF cell counts Lung tissue cytokines, neuropeptides, and histology/IHC	Total cells, eosinophils, and lymphocytes were increased in BALF by FA alone, and all of these cells (minus lymphocytes but plus neutrophils) were increased more robustly by FA+ OVA Histopathology: increased inflammation FA increased lung IL-4, IL-1β, substance P, and CGRP, but not IFNγ; more robustly by FA+OVA (peptide changes by IHC also) TRPA1 and TRPV1 antagonists reduced FA+OVA-induced eosinophil counts (anti-TRPA1 also decreased neutrophils), and lung factors (except IL-1)	**Low Confidence [formalin; pharmacological interventions did not include effects of FA alone]**
		Sensitization: s.c. 80 µg OVA on Days 10, 18, and 25 Challenge: 1% OVA aerosol 30 min/d on Days 29–35			
(Qiao et al., 2009)	Male Wistar rats (n=8/group)	Formalin 0, 0.51 or 3.08 mg/m^3 for 3 wk (6 hr/d)	BALF cell counts Lung histology and cytokine levels	"slight but insignificant pulmonary abnormalities" with FA alone; OVA 3.18 mg/m^3 changed airway structure N/C in BAL total cells or eosinophils with 3.18 mg/m^3, but ≥0.51 mg/m^3 dose-dependently increased both in presence of OVA; 3.18 mg/m^3 FA alone increased IFNγ and decreased IL-4; FA+OVA increased IL-4	**Low Confidence [formalin]**
		Sensitization: i.p. OVA on Days 10 and 18 Challenge: 1% OVA 30 min/d for 7 d			
	Male Balb/c mice (n=6/ group)	Formalin 0, 0.5, or 3 mg/m^3 for 21 d (6 hr/d)	BALF cell counts	Cell infiltration and airway remodeling in 3 mg/m^3 FA + OVA	**Low Confidence [formalin]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Liu et al., 2011)		Sensitization: i.v. 20 mg OVA on Day 10 and 1 Challenge: 1% OVA aerosol for 30 min/d for 7 d	Pulmonary histology and cytokines	Increased % Eosinophils at ≥ 0.5 mg/m^3, which is amplified by OVA; N/C IFNγ Increased lung IL-4 and IL-6 at 3 mg/m^3; with OVA, this is observed at 0.5 mg/m^3	
(Ye et al., 2013)	Male Balb/c mice (n≥9/ group/ endpoint)	Formalin 0, 0.5, 1, or 3 mg/m^3 for 7 d (8 hr/d)	ROS (dichlorohydro-flourescein and MDA) and GSH in Lung	Dose-dependent decrease in GSH levels in lung at ≥0.5 mg/m^3 Dose-dependent increase in DCFH and MDA in lung at ≥1 mg/m^3 Co-administered GSH attenuated effects	**Low Confidence [formalin]**
(Abreu et al., 2016)	C57BL/6 mice (n=12 M+F/ treatment group and n=6 M+F/ control)	Formalin (assumed) 0, 0.25, 1.2, and 3.7 mg/m^3 for 8 hr (aldehyde mixture data not included herein; authors noted some exposure cross-contamination)	Lung histology (cells and morphology) (blinded measures 6–8 hr postexposure) Lung cytokine, catalase, and SOD levels/ activity	FA increased distended alveoli at 3.7 mg/m^3; N/C in total mononuclear or polymorphonuclear cells N/C in IL-1, IL-6, TNF, CCL2, or MIP-2, or in antioxidants; increased keratinocyte chemoattractant at 0.25 mg/m^3 only Note: N/C in lung mechanics except increased airway inertance (might indicate an impedence of airflow) at 3.7 mg/m^3	**Low Confidence [formalin; short duration and periodicity; some coexposure to acetaldehyde possible- unclear]** Note: ACUTE
(Sandikci et al., 2007b)	SD rats (n=6/ group) at GD1 [I], PND1 [II], PND28 [III] or adults [IV]	Formalin (assumed: test article NR): 0 or 7.38 mg/m^3 for 6 wks (8 hr/d, 7 d/wk)	BALT T lymphocyte CD4+, CD8+ counts (by IHC)	Increased BALT T lymphocytes (ANAE+ as marker); CD4+ T cell counts and size of BALT increased in Groups III and IV; CD8+ T cell counts increased in Group III Note: body weight was significantly decreased in Groups I and II	**Low Confidence [formalin; high exposure levels]** Note: limited assays
(Sandikci et al., 2007a)	Female SD rats at GD1 [i], PND1 [ii], PND 28 [iii], or PND90 [iv] (n=6)	Formalin (assumed; test article NR) 0, 7.38 mg/m^3 for 6 wks (8 hr/d, 7 d/wk)	BALT T lymphocyte counts; BALT size Note: body weight decreased by FA in groups i and ii	CD4+ cell counts increased in groups iii and iv; CD8+ cell counts increased in group iii (group iv N/S increased) Increased size of BALT in adults (iii & iv)	**Low Confidence [formalin; high exposure levels]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Jung et al., 2007)	Female C57BL/6 mice (n=10/ group)	Formalin (assumed; test article NR) 0, 6.15, 12.3 mg/m^3 for 2 wk (6 hr/d, 5 d/wk)	Lung oxidative stress (intracellular, by flow) BAL and lung homogenate counts, and histopath. Cytokine mRNA and protein	Oxidative stress (DCFH-DA) at ≥6.15 mg/m^3 Total BAL cells increased (2-fold) at 12.3 mg/m^3; Slight changes in B220+ B cells (↓) and CD3+ or CD4+ T cells (↑) were not interpreted as significant; CD8+ T cells were ↑, only slightly; N/C in neutrophils Large increase in eosinophil counts from BAL, and in flow counts and gene expression of lung tissue at 12.3 mg/m^3, eosinophil infiltration, and epithelial damage, by histopath at ≥6.15 mg/m^3 Increased IL-4, IL-5, and IL-1β (not IL-13) in lung at 6.15 and 12.3 mg/m^3 body weights decreased ≈10%	**Low Confidence [formalin; high exposure levels; statistical significance of flow data NR]** Note: Th2 cytokines
(Sul et al., 2007)	Male SD rats (n=10/group)	Formalin (assumed; test article NR) 0, 6.15, 12.3 mg/m^3 for 2 wks	Lung tissue oxidative stress and mRNA array	Lipid peroxidation (MDA) and protein oxidation were increased at 12.3 mg/m^3 Changes in 21 genes, including D/D decrease in 3 immune-related genes: HSP70$_{1a}$, complement 4 binding protein, and Fc receptor IgG low affinity III	**Low Confidence [formalin; high levels]** NOTE: utility of mRNA results by themselves unclear
(Lu et al., 2005)	Male Kun Ming mice (n=5)	Formalin 0, 0.5, 1, or 3 mg/m^3 for 10 d (6 hr/d)	BALF IL-4 (undetected in serum)	D/D Increased IL-4 at ≥1 mg/m^3 FA Blocked by vanilloid (TRPV) receptor antagonist, CPZ	**Low Confidence [formalin; small sample size]**
(Ahn et al., 2010)	Male SD rats (n=4/group)	Formalin (assumed; test article NR) 0, 2.46, or 24.6 mg/m^3 for 2 wk (6 hr/d)	BAL fluid proteomic analysis	6 proteins increased (3 inflammatory serpins, anti-inflammatory annexin, an erythrocyte protein associated with trauma or inflammation, and a metabolic enzyme); 5 proteins were decreased	**Low Confidence [formalin]** NOTE: unclear utility of measures

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Kimura et al., 2010)	Male Wistar (n=5−6)	Formalin 1.23, 6.15, 18.5, or 55.4 mg/m^3 for up to 45 min	Airway microvascular leakage (lung- main bronchi and trachea) BALF counts of leukocytes Shed epithelial cells in BALF	D/D increase leakage by 15 min at ≥ 1.23 mg/m^3; not exacerbated with longer/ repeated exposure Note: Leakage induced by substance P was not inhibited by pre-FA exposure, but preinhalation of the same mg/m^3 abolished FA-induced leakage and pre-FA inhibited capsaicin-induced leakage; however, 20 hr between exposures allows for recovery of tachykinins and leakage by FA exposure Inhibition of mast cell activation (H1 receptor antagonist), but not cyclooxygenase products (indomethacin), blocked FA leakage at 6.15 mg/m^3; increased shed epithelial cells 20 hr, but not immediately, after 6.15 mg/m^3 for 30 min Increased BALF neutrophils with preinhalation at 6.15 mg/m^3, but N/C eosinophils or mononuclear cells	**Low Confidence [formalin; small sample size; short duration]** Note: Authors hypothesize preinhalation of FA depletes the amount of tachykinins available at the target site (but not desensitization of NK1 receptors), in part b/c capsaicin can no longer induce a response; also, because of recovery, up to 6.15 mg/m^3 does not cause irreversible damage to airway sensory nerves, but that prolonged exposure (≥7 d) might exacerbate neurogenic airway inflammation
(Dallas et al., 1987)	Male SD rats (n=2/ timepoint; unclear reporting)	PFA 0, 0.62, 3.69, or 18.5 mg/m^3 for 1 wk to 24 wk (6 hr/d, 5 d/wk)	Flow cytometry DNA/RNA analysis of alveolar cell proliferation/ health	Increased RNA index in alveolar cells at all FA levels at 1 wk; only at ≥ 3.69 mg/m^3 at 8 wk; N/C in DNA (e.g., % S phase) [Note: same alveolar samples had chromatid breaks at 18.5 mg/m^3]	**Low Confidence [small sample size; unclear reporting]** NOTE: unclear specificity/ utility of methods
(Kim et al., 2013a)	Female C57BL/6 mice (n=5 "experiments"; number of mice/ group unclear)	Formalin (assumed; test article NR) 0, 6.15, or 12.3 mg/m^3 for 2−3 wk (6 hr/d, 5 d/wk)	Lung cell counts BAL cell counts Ex vivo cellular functional assays	N/C in lung tissue total cells, but number of NK1 cells markedly decreased (this recovered by 2 wks postexposure) at 12.3 mg/m^3 Lung NK1 cell mRNA and protein markers (IFNγ, perforin, and CD122) were D/D decreased at ≥ 6.15 mg/m^3 BAL total cells increased, but number of NK cells decreased at 12.3 mg/m^3	**Low Confidence [formalin; high levels; small sample size]** **Not Informative: ex vivo experiments or in vitro FA treatment of NK precursors showing reduced differentiation to mature cells**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
				N/C in other lung or BAL lymphocyte populations (e.g., % CD4+ or CD8+ cells)	
(Sadakane et al., 2002)	Male ICR mice (n=9 or 18)	Formalin 0.5% for 4 wk (15 min/wk) Sensitization: i.p. with 3 mg/mL Der f (house dust mite allergen) prior to FA Challenge: intratracheal 10 µg Der f 3 hr after last exposure (note: measures 3 d later)	Lung IHC cell counts and cytokine analysis	N/C in lung eosinophil recruitment or goblet cell proliferation by FA alone, but Der f-induced eosinophil recruitment was exacerbated by FA Increased RANTES in lung by FA alone, and exacerbated increase to Der f-changes with FA for IL-5 and RANTES; N/C in lung IL-2 or IL-4	**Low Confidence [formalin; unquantified high levels; short periodicity]**
(Sandikci et al., 2007a)	Female SD rats at PND1, PND28, or PND90 (n=3)	Formalin (assumed; test article NR) 0 or 7.38 mg/m^3 for 6 wk (8 hr/d, 7 d/wk)	Lung and BALT histology	N/C in exposed PND1 group Increased apoptotic cells in lungs and BALT of PND28 and PND90 groups Authors: apop. cells likely lymphocytes	**Low Confidence [formalin; high level; small sample size]**
(Matsuoka et al., 2010)	Male ICR mice (n≥ 7)	Formalin at 0.12 mg/m^3 for up to 24 hr; also, a single experiment at 3.69 mg/m^3 for 24 hr	lung ROS (8OHdG) and NO metabolites (nitrates/ nitrites); at 3.69 mg/m^3: LPS response	Decreased ROS lung; N/C in NOs or lung NOs after LPS injection	**Low Confidence [formalin; short duration]** NOTE: ACUTE
(Yan et al., 2005)	Male Kun Ming mice (n=6)	Mixture (test article wood panels) 0, 0.5, 1, or 3 mg/m^3 for 72 hr (24 hr/d)	Lung NOS activity and NO measurement	Increased NOS activity at 3 mg/m^3 FA (p = 0.06 at 1 mg/m^3) NO was detected more frequently in samples from 3 mg/m^3 FA group (50% vs. 17%)	**Low Confidence [wood panel exposure; lack of controls for co-exposure; short duration]** NOTE: NO detection did not include statistical comparisons
(Dinsdale et al., 1993)	Male SD rats (n=4, 6, or 10)	PFA or Formalin 12.3 mg/m^3 for 4 d (6 hr/d)	Lung enzymes (in BAL or tissue) Lung histology	Increased cytochrome P450 and decreased γ-glutamyl transpeptidase with PFA exposure (not with formalin) No abnormalities (i.e., signs of injury or repair) by histology	**Low Confidence [small sample size; excessively high levels; short duration]** NOTE: Endpoints not very informative for inflammation (injury response, possibly)
(Rager et al., 2011)	In vitro (human lung cancer cell line); n=6 replicates	PFA 1.23 mg/m^3 for 4 hr or air controls	In vitro epithelial cell miRNA microarray and IL-8 secretion	Increased IL-8 release >16-fold with FA 89 miRNAs were downregulated by FA; the 4 most robust were associated with inflammatory response pathways	**Low Confidence [in vitro; short duration; exposure level comparability to inhalation unclear]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Zhao et al., 2020)	Male Balb/c mice (n=3, pooled into single sample for nose and lung samples); 2 experiments by different researchers	Formalin 0, 3 mg/m^3 for 2 wks (8 hr/d, 5 d/wk)	Burst-forming unit-erythroid (BFU-E), and colony-forming unit-granulocyte macrophage (CFU-GM) colonies in nose, lung, spleen, and bone marrow	Lung (ex vivo) results: Decreased formation of BFU-E in experiment II; N/C in experiment I Decreased formation of CFU-GM in experiment II; N/C in experiment I Lung (in vitro treatment): Up to 400 uM formaldehyde caused N/C in BFU-E not CFU-GM formation	**Low Confidence [formalin; small sample size; in vitro (for cell treatments)]**
(Maiellaro et al., 2014)	Pregnant Wistar rats (n=5; note: individual pup data for n=10 pups did not appear to account for litters)	Formalin 0.92 mg/m^3 from GD1-GD21: 1 hr/d, 5 d/ wk Sensitization: s.c. 10 µg OVA with sc boost after 7d Challenge: 7 d later, 1% OVA aerosol 15 min/d, 3d	BAL cell counts and factors Lung factors	N/C in parental BAL total cells, monocytes, lymphocytes, or granulocytes N/C in parental lung IL-4, IL-6 or IL-10; Decreased birth weight in offspring 24 hr after OVA challenge, offspring have: decreased BAL total cells, mononuclear cells, neutrophils, and eosinophils; Increased BAL IL-10, but decreased IL-6 and TNFα (N/C in IL-4)	**Not Informative [formalin, short periodicity; small sample size; offspring comparisons do not include FA alone; did not appear to account for litter effects]**
(Maiellaro et al., 2016)	Pregnant Wistar rats (n=5 dams; note: individual pup data for n=10 pups did not appear to account for litters)	Formalin 6.13 mg/m^3 from GD1-GD21: 1 hr/d, 5 d/wk Sensitization on PND 30: s.c. 10 µg OVA Challenge: 14d later, 1% OVA aerosol 15 min/d, 3 d	BAL cell counts and factors in pups on ≈PND45	Increased (amplified) total BAL leukocytes Increased (amplified) BAL mononuclear cells and neutrophils Increased (amplified) myeloperoxidase Decreased (slightly reduced) eosinophils and eosinophil peroxidase	**Not Informative [formalin, short periodicity; small sample size; offspring comparisons do not include FA alone; did not appear to account for litter effects]**
(Silva Ibrahim et al., 2015)	Pregnant Wistar rats (n=5 dams; 10 pups/group for experiments; note: individual pup data for n=10 pups did not	Formalin 0.92 mg/m^3 from GDs 1–21: 1 hr/d, 5 d/wk	Cell number, cytokine and neutrophil marker (MPO) in BAL Function of BAL cells Lung gene and proteins	24 hr after LPS challenge, offspring exposed to formaldehyde have reduced immune responses to LPS (i.e. decreased BAL cells and granulocytes-N/C in lymphocytes or monocytes; decreased MPO and oxidative burst-N/C in phagocytosis; decreased IL-6	**Not Informative [formalin; short periodicity; offspring comparisons do not include FA without LPS; small sample size; did not appear to account for litter effects]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
	appear to account for litters)	Randomly assigned pups all received 5 mg/kg lipopolysacharride (LPS) injections at PND 30		and increased IFN and IL-10; decreased TLR4 and NFkB)	
(Ibrahim et al., 2016)	Pregnant Wistar rats (n=5 dams; 10 pups/ group for experiments; note: individual pup data for n=10 pups did not appear to account for litters)	Formalin 0.92 mg/m^3 from GDs 1−21: 1 hr/d, 5 d/wk	Total BAL cell number and cytokine gene expression	Increased cell number by LPS was reduced in offspring exposed to formaldehyde Formaldehyde increased IFN expression, decreased IL-6, TLR4, and NF-kB expression, and caused N/C in IL-10, as compared to LPS	**Not Informative [formalin; short periodicity; offspring comparisons do not include FA without LPS; small sample size; did not appear to account for litter effects]** **Note: effects rescued by vitamin C**
		Randomly assigned pups all received 5 mg/kg lipopolysacharride (LPS) injections at PND 30			
(da Silva et al., 2015)	Male Wistar rats (n=6/ group)	Formalin 1% for 3 d (90 min/d); rats exposed in static chambers 5 rats/ time	BAL cell counts Lung vascular permeability BAL and lung cytokines (all measures at 24 h postexposure except permeability, which was immediate)	FA increased total BAL cells, activated mast cells, and neutrophils (latter based on myeloperoxidase activity) FA did not change trachea permeability (Evans blue), but did increase it in lung parenchyma and bronchii FA increased TNF, IL_6, and N/C IL-10 in BAL, and increased IL-10, but not IL-6 mRNA in lung tissue Note: while reduced effects were reported as reduced with laser therapy, laser therapy-only controls were not used	**Not Informative [formalin; unquantified high levels; static exposure chamber and group exposure; short duration and periodicity]**
(Murta et al., 2016)	Male Fischer rats (n=7)	Formalin (assumed) 1%, 5%, or 10% for 5 d (3 × 20 min/d)	BAL cell counts Lung histopathology and chemokine levels	FA increased total leukocyte, macrophages at 10%, and lymphocytes at ≥5%; N/C in neutrophils or eosinophils; ≥5% caused lung parenchyma damage; ≥1% increased CCL5 and 10% CCL2 (N/C in CCL3)	**Not Informative [formalin; unquantified high levels; static exposure chamber; short periodicity]**
(Kilburn and Mckenzie, 1978)	Male and female Syrian golden hamster (n=6−14)	PFA "low": 3.69 or 7.38 mg/m^3 or "high": ≥246 mg/m^3 for 4 hr; alone,	Lower airway PMN Leukocyte recruitment and	Although cytotoxic effects were observed at ≥3.69 mg/m^3, FA alone did not induce PMN leukocyte	**Not Informative [short duration, precision of exposure levels unclear; reporting**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
		with carbon dust, or evaporated onto carbon	cellular changes by histology	recruitment; FA + carbon caused leukocyte recruitment 2 hr postexposure, which peaked at ≈20 hr and resolved by 1 wk; recruitment was similar at "low" and "high" levels	**difficult to follow, and data NR for all exposure levels indicated as tested; nonexposed controls did not appear to be included]**
(Persoz et al., 2010)	In vitro (human immortalized lung cells); n=4 experiments	Formalin gas: 0.050 mg/m^3 for 30 min, ± TNFα sensitization	Lung cell Cytokine secretion (at 24 hr post-FA)	N/C in IL-6, IL-8, or MCP-1 without TNF α sensitization Increased IL-8 only with sensitization Note: air exposure alone increased IL-8	**Not Informative [formalin; in vitro; short duration; unknown exposure level relevance; small sample size; controls exhibited effects from air-only exposure]**
(Persoz et al., 2011)	In vitro (human immortalized lung cells); n=4 experiments	Formalin gas: 0.050 mg/m^3 for 30 min, with or without aspergillus spores (Asp)	Lung cell cytokine secretion (at 24 hr post-FA)	N/C in IL-8 or MCP-1 mRNA or protein	**Not Informative [formalin; in vitro; short duration; unknown exposure level relevance; small sample size; controls exhibited effects from air-only exposure]**
(Persoz et al., 2012)	In vitro (human immortalized lung cells); n≥3 experiments	Formalin gas: 0.050 mg/m^3 for 30 min; treatment with sensitizers (i.e., TNFα or MCM)	Bronchial or alveolar cytokine secretion (at 24 hr post-FA)	IL-8 production in alveolar cells induced by TNFα or macrophage-conditioned media (MCM) increased by FA MCP-1 production in bronchial cells induced by sensitizers increased by FA N/C om IL-8 or MCP-1 otherwise Note: expression affected by air alone	**Not Informative [formalin; in vitro; short duration; unknown exposure level relevance; small sample size; controls exhibited effects from air-only exposure]**
(Kastner et al., 2013)	In vitro (human immortalized lung cells); n=3 experiments	Formalin gas: 0.2 mg/m^3 for 30 min, 1 hr, or 2 hr/day once or for 4 d	Lung cell cytokine secretion and epithelial barrier function/ viability (at 24 hr post-FA)	N/C in IL-6 or IL-8 release, or TEER (measures disruption to epithelial cell monolayer) by FA alone Note: viability affected by air exposure	**Not Informative [formalin; in vitro; short duration; unknown exposure level relevance; small sample size; controls exhibited effects from air-only exposure]**
(Lino-Dos-Santos-Franco et al., 2013a)	Female Wistar rats (n=5)	Formalin 1% or methanol vehicle for 3 d (90 min/ d), ± ovariectomy	BAL counts Ex vivo lung IL-10	1 d after challenge: FA/OVA versus OVA alone decreased total cell counts, including mononuclear cells, neutrophils, and eosinophils FA/OVA versus OVA alone: Robust IL-10 increase	**Not Informative [formalin (MeOH controls); naïve not chamber exposed; unquantified high levels; FA alone untested; small sample size]**
		Sensitization: After FA, s.c. 10 µg OVA, with s.c. boost 7 d later Challenge: After 7 d, 1% OVA aerosol for 15 min			

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Lino-Dos-Santos-Franco et al., 2010)	Male Wistar rats (n=5-6)	Formalin 1% for 3 d (90 min/d)	Lung cellular oxidative burst (flow) and tissue oxidative stress-peroxynitrite (3-NT)	Increased cellular oxidative burst (DFFH, ± OVA) Increased lung nitration (peroxynitrite formation; without OVA)	**Not Informative [formalin; unquantified high levels; small sample size; short duration and periodicity]** Note: vitamin C, E blunted effects
		Sensitization: immediately post-FA, s.c. 10 µg OVA; boost 1 wk later with s.c. 10 µg OVA injection Challenge: 1 wk later with 1% aerosol OVA (15 min)			
(Macedo et al., 2016a)	Male Wistar rats (n=6)	Formalin 1% for 3 d (90 min/d)	Lung (or lung cells) oxidative stress indicators: H_2O_2, nitrites, oxidative burst, enzyme activity and gene expression of redox-related proteins	Formaldehyde exposure increased H_2O_2 and NO_2, but not DCFH-DA (oxidative burst), and exposure increased expression of cNOS and iNOS, SOD and catalase, but did not affect the activity of enzymes associated with detoxification processes (e.g., glutathione reductase)	**Not Informative [formalin; unquantified high levels; short duration and periodicity]** **Note: Photobiomodulation (laser) therapy blunted effects**
(Lima et al., 2015)	Male Fischer rats (n=7)	Formalin 1, 5, or 10% for 5 d (20 min × 3/d)	Trachea or diaphragm muscle (DM) oxidative stress indicators: carbonyl protein, lipid peroxidation, and catalase activity; and inflammatory cell influx	In Trachea: increased lipid peroxidation at 1 and 5, but not 10%; N/C in catalase or inflammatory cell influx; increased mucus deposits at 5%, and increased metaplasia and ulceration at 10% In DM: increased lipid peroxidation at 1 and 5, but not 10%; increased carbonyl protein and increased inflammatory cell influx at 10%; decreased catalase at ≥1%	**Not Informative [formalin; unquantified high levels; short duration and periodicity; controls not chamber exposed]**
(Lino dos Santos Franco et al., 2009)	Male Wistar rats (n=5)	Formalin 0, 1% for 3 d (90 min/d)	BAL nitrites	FA increased BAL nitrites, which was exacerbated with OVA sensitization	**NotInformative [formalin; unquantified high levels; small sample size; short duration and periodicity]**
		Sensitization: immediately post-FA, i.p. 10 µg OVA; boost 1 wk later with s.c. injection Challenge: 1 wk later with aerosolized OVA			

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Lino-Dos-Santos-Franco et al., 2013b)	Male Wistar rats (n=5–8)	Formalin 1% or naive for 3 d (90 min/ d), with or without subsequent OVA Sensitization: after FA inhalation, s.c. 10ug OVA with same boost 7 d later Challenge: after 1 wk, 1% OVA aerosol for 15 min	Lung mRNA Ex vivo Lung factors	FA increased iNOS and COX-1, but not COX-2, expression in lung (OVA and FA seemed to attenuate induction by other) FA/OVA vs. OVA increased NO and LTB_4 (both inhibited by inhibition of NOS or by inhibition of COX), but not TXB_2 or PGE_2 Note: suggests mast cell- and NO-mediated effects	**NotInformative [formalin; unquantified high levels; small sample size; short duration and periodicity; comparisons reported did not include all relevant controls (e.g., FA alone; air alone)]**
(Lino-Dos-Santos-Franco et al., 2011b)	Male Wistar rats (n=5/ group)	Formalin 1% for 3 d (90 min/d)	BAL cell counts Lung ROS Ex vivo lung cytokines in explants or cultured BAL cells	FA increased total BAL cells, mononuclear cells, and neutrophils FA decreased SOD, but not catalase, GPX, GR, or GST activity in lung tissue; mRNA expression for SOD, catalase, NOS, and COX was increased FA increased IL-1β and IL-6 in explants; increased NO_2 and H_2O_2 in BAL cells	**NotInformative [formalin; unquantified high levels; small sample size; short duration and periodicity; some ex vivo]**
(Lino dos Santos Franco et al., 2006)	Male Wistar (n=5-6)	Formalin 1% or methanol vehicle for 4 d (30, 60, or 90 min/d)	BAL cell counts Lung IHC Ex vivo BAL nitrites	Increased BAL Total cells (90 min only), mononuclear cells (60 and 90 min), and neutrophils (30, 60, or 90 min) Increased ex vivo cultured BAL cell release of nitrites Lung IHC showed mast cell degranulation and neutrophil infiltration Note: number of cells recovered in BAL was significantly reduced by capsaicin (depletes neuropeptides from sensory nerve endings), but bronchial hyporesponsiveness not altered; conversely L-NAME (inhibits NO synthase) did not affect BAL cells, but did restore bronchial responsiveness; administration of 48/80 to deplete mast cells blunted FA-induced effects	**Not Informative [formalin ((MeOH controls); unquantified high levels; small sample size; short duration and periodicity; comparisons reported to naïve rats rather than MeOH controls; some ex vivo]** NOTE: if a relevant MOA is identified from more informative studies, pharmacological intervention endpoints might be reconsidered

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
				on both BAL cell counts and bronchial response	
(Lino-Dos-Santos-Franco et al., 2011a)	Female Wistar rats (n=5)	Formalin 1% or naïve for 3 d (90 min/d), with or without ovariectomy	BAL counts and mast cell degranulation	FA increased total BAL cell counts, mononuclear cells and neutrophils, but not eosinophils Decreased lung mast cell number and increased degranulation	**Not Informative [formalin; unquantified high levels; small sample size; short duration and periodicity; impact of sham surgery/ FA alone untested; naïve not chamber exposed]**
(Lino-Dos-Santos-Franco et al., 2010)	Male Wistar rats (n=5-6)	Formalin 1% for 3 d (90 min/d) Sensitization: immediately post-FA, s.c. 10 µg OVA; boost 1 wk later with s.c. 10 µg OVA injection Challenge: 1 wk later with 1% aerosol OVA (15 min)	Pulmonary vascular permeability (Evans blue) BAL cell counts Ex vivo cultured BAL cells factors/cytokines Phagocytosis (flow)	Increased BAL mononuclear cells and neutrophils, but N/C in eosinophils or in lung ICAM-1 Increased vascular permeability (± OVA) FA increased ex vivo LTB4; FA+OVA increased BAL LTB4, TXB2, IL-1b,Il-6,VEGF N/C in phagocytosis;	**Not Informative [formalin; unquantified high levels; small sample size; short duration and periodicity; some ex vivo]** Note: vitamin C and E blunted effects
(Kita et al., 2003)	Male Hartley guinea pigs (n=10+/group)	Nasal Instillation of saline or Formalin 0.1 or 1.0%; 3×/wk for 6 wk Sensitization: intradermal anti-OVA serum on day 38 (passive) or i.p. 2 mg OVA on Day 3 (active) with boost i.p. 10 mg OVA day 24 Challenge: 1 mg/mL nebulized OVA 15 min after last FA exposure on day 45	BAL cell counts	N/C in BAL fluid cell counts by FA with passive or active sensitization (not measured for FA alone)	**Not Informative [formalin; high, unknown levels; short periodicity; exposure route; effect of FA alone not measured]**
(Kita and Oomichi, 1974)	In/Ex vitro: trachea from guinea pigs (n=3)	Formalin gas: 39.4 or 67.7 mg/m^3 for <30 min	In vitro ciliary beat frequency	FA decreased CBF 50% in 11.5 min (39.4 mg/m^3) or 4.5 min (67.7 mg/m^3)	**Not Informative [formalin; excessively high levels; short duration; ex vitro; small sample size]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Lino dos Santos Franco et al., 2009)	Male Wistar rats (n=5)	Formalin 0, 1% for 3 d (90 min/d)	BAL cell counts Lung mast cell degranulation	Increased Total BAL cells, mononuclear cells, and neutrophils (eosinophils undetected); FA inhibited OVA-induced increases in all cell counts	**Not Informative [formalin; unquantified high levels; small sample size; short duration and periodicity]**
		Sensitization: immediately post-FA, i.p. 10ug OVA; boost 1 wk later with s.c. injection Challenge: 1 wk later 1% aerosol OVA for 15 min		FA increased mast cell degranulation; FA inhibited OVA induced degranulation FA induced PECAM expression; FA inhibited OVA induced increases	

Table A-68. Changes in pulmonary function involving provocation (e.g., bronchoconstrictors; allergens; etc.)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
Observational Epidemiology Studies					
(Górski and Krakowiak, 1991)	Human textile and shoemakers (n=367)	Not exceeding 0.5 mg/m^3 (duration at least 1 yr (average= ≈12 yrs)	Bronchial hyper-reactivity to histamine	Bronchial hyperreactivity in 11 nonbronchitic patients (14 bronchitic/2 asthmatic ppl)	**Low Confidence [incomplete and confusing methods and results; comparisons unclear]**
Controlled-Exposure Studies in Humans or Primary Human Cells					
(Krakowiak et al., 1998)	Human workers with bronchial asthma or healthy subjects (n=10 each)	Formalin (assumed: test article NR): 0.5 mg/m^3 for 2 hr with follow-up out to 24 hr	Bronchial provocation responses (histamine)	N/C in Bronchial reactivity to histamine (Note: scoring measures of nasal symptoms were elevated)	**Low Confidence [formalin; short duration; small sample size]** NOTE: ACUTE; no effect on FEV_1, etc.
(Casset et al., 2006)	Human (n=19 with mild asthma and allergy to mite allergen)	Formalin ≈0.1 mg/m^3 for 30 min; placebo at ≈0.03 mg/m^3 double-blind randomized; restricted to mouth breathing only	Airway response to mite allergen (Note: large allergen size chosen to deposit in large airways)	A lower level of allergen was necessary to induce bronchoconstriction following FA exposure and FA exposure: both immediate and late-phase responses; note: N/C in pulmonary function tests with FA exposure alone prior to allergen challenge	**Low Confidence [formalin; short duration; not clear that restriction to mouth breathing is realistic for typical inhalation]** NOTE: ACUTE; within-subjects comparison between air and FA
(Ezratty et al., 2007)	Human (n=12 intermittent asthmatics with allergy to pollen)	Formalin 0.5 mg/m^3 for 60 min; randomized to air or FA first (no nonexposed controls)	Allergen (pollen)-induced changes in airway FEV1 and MCh responses (note: did	N/C in pulmonary function by allergen (a borderline decreased response, p = 0.06, was observed) or to MCh responsiveness after allergen	**Low Confidence [formalin; short duration]** NOTE: ACUTE; within subjects comparison between air and FA

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
			not appear to test MCh w/o allergen) 8 hr later	challenge; note: N/C in pulmonary function by FA	
Controlled-Exposure Studies in Animals, Animal Cells, or Immortalized Human Cells					
(Riedel et al., 1996)	Female Dunkin-Hartley guinea pigs (n=12)	Formaldehyde (bottled pressurized gas) 0, 0.16, 0.31 mg/m^3 for 5 d (8 hr/d) Sensitization: 0.5% inhaled OVA; OVA boost at 2wk Challenge: 1% inhaled OVA 1 wk later	Airway response to OVA	Increased OVA challenge-induced airway obstruction by 0.31 mg/m^3 (3, 7, and 10 animals exhibited airway obstruction across groups)	**High or Medium Confidence [no comparison group with FA without OVA]** NOTE: guinea pigs have been shown to be more sensitive to airway constriction from toxicants than other animals]
(Leikauf, 1992) [considered same cohort as (Swiecichowski et al., 1993)]	Male Hartley guinea pigs (n=5-7)	PFA 0, 0.12, 0.37, 1.23, 3.69, 12.3, or 36.9 mg/m^3 for up to 8 hr	Bronchial reactivity to i.v. acetylcholine	Increased specific resistance at ≥12.3 mg/m^3 with 2 hr; Increased at ≥1.23 mg/m^3 with 8 hr (i.e., duration > concentration); with 8 hr, hyperreactivity persisted >24 hr postexposure	**See Swiechichowski et al., 1993** NOTE: ACUTE
(Swiecichowski et al., 1993)	Male Hartley guinea pigs (n=5−7/group)	PFA from 0.12−123 mg/m^3, for 2 or 8 hrs (multiple experiments)	Airway reactivity Ex vivo airway reactivity (trachea)	Increased pulmonary resistance (reversible bronchoconstriction) and airway reactivity to acetylcholine at ≥1.23 mg/m^3 (not at 0.36 mg/m^3) for 8 hr; at ≥ 12.3 mg/m^3 (not at ≤3.6 mg/m^3) for 2 hr Increased ex vivo reactivity (smooth muscle contraction) at 4.18 mg/m^3 for 8 hr	**High or Medium Confidence at 1.23 mg/m^3 and above [short duration]** **Low Confidence below 1.23 mg/m^3 and ex vivo [ex vivo; sample size of 5 at 1 or more levels below 1ppm]** NOTE: ACUTE; duration appeared to be more important than FA level for pulmonary resistance
(Larsen et al., 2013)	Male BALB/cA mice (n=10)	PFA 0.49, 2.21, or 4.9-7.0 (dry vs. humid air) mg/m^3; 60 min	Airway reactivity	Increased airway reactivity (decreased expiratory flow rate) in humid air in OVA-sensitized mice at 7 mg/m^3	**High or Medium Confidence [short duration]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
		Sensitization: pre-FA i.p. 1 µg OVA, with 0.1 µg OVA boosts i.p. on days 14 and 21 (note: FA on day 31) Challenge: 0.2% OVA aerosol- 20 min on day 29 and 30		Increased bronchoconstriction in a dry environment without OVA sensitization at 4.92–7.0 mg/m^3 (with OVA sensitization reducing the response to formaldehyde)	NOTE: ACUTE; suggests that environmental humidity may affect acute airway reactivity induced by formaldehyde; experiments on inflammatory markers (below) considered less informative
(Liu et al., 2011)	Male Balb/c mice (n=6/ group)	Formalin 0, 0.5, or 3 mg/m^3 for 21 d (6 hr/d)	Airway reactivity	Slightly increased responsivity to MCh compared to saline controls; robust amplification in 3mg/m^3 FA+OVA group	**Low Confidence [formalin]**
		Sensitization: i.v. 20 mg OVA on Days 10 and 21 Challenge: 1% OVA aerosol for 30 min/d for 7 d			
(Qiao et al., 2009)	Male Wistar rats (n=8/group)	Formalin 0, 0.51 or 3.08 mg/m^3 for 3 wk (6 hr/d)	Airway response to methylcholine	3.08 mg/m^3 FA alone increased hyperresponsiveness to MCh, which was amplified with OVA administration at ≥ 0.51 mg/m^3	**Low Confidence [formalin]**
		Sensitization: i.p. OVA on Days 10 and 18 Challenge: 1% OVA 30 min/d for 7 d			
(Wu et al., 2013)	Male Balb/c mice (n=8/group)	Formalin 0, 3 mg/m^3 for 4 wk (6 hr/d, 5 d/wk)	Airway responsivity to Methylcholine (MCh)	Airway was slightly hyperesponsive to MCh by FA alone, but severely so in FA+OVA groups TRPA1 and TRPV1 antagonists reduced FA+OVA-induced airway responsiveness	**Low Confidence [formalin; pharmacological interventions did not include effects of FA alone]**
		Sensitization: s.c. 80 µg OVA on days 10, 18, and 25 Challenge: 1% OVA aerosol 30 min/d on Days 29–35			
(Biagini et al., 1989)	Male cynomolgus monkeys (n=9)	Formalin 3.08 mg/m^3 for 10 min (challenge experiment)	Bronchoreactivity to methylcholine (all with MCh)	Increased bronchoconstriction by FA challenge at 2, 5, and 10 min postchallenge	**Low Confidence [formalin; short duration; FA without methylcholine untested]**
(Maiellaro et al., 2014)	Pregnant Wistar rats (n=5)	Formalin 0.92 mg/m^3 from GDs 1–21: 1 hr/d, 5 d/wk	Tracheal response to MCh	24hr after OVA challenge, offspring have: decreased tracheal response to MCh Note: Decreased birth weight in offspring. Nonmanipulated group exhibits large, unexplained differences from vehicle control (and has reporting limitations)	**Not Informative [formalin; short periodicity; offspring comparisons do not include FA alone; unclear comparability for some groups; small sample size]**
		Sensitization: s.c. 10 µ g OVA with sc boost after 7 d Challenge: 7 d later, 1% OVA aerosol 15 min/d, 3 d			
	Pregnant Wistar rats (n=5 dams;	Formalin 0.92 mg/m3 from GDs 1–21: 1 hr/d, 5 d/wk	Response to MCh	24 hr after LPS challenge, offspring exposed to formaldehyde have decreased MCh response	**Not Informative [formalin; short periodicity; offspring**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Silva Ibrahim et al., 2015)	10 pups/ group for experiments)	Randomly assigned pups all received 5 mg/kg lipopolysacharride (LPS) injections at PND 30			**comparisons do not include FA without LPS; small sample size]**
(Kita et al., 2003)	Male Hartley guinea pigs (n=5−7/group)	Nasal Instillation of saline or Formalin 0.1 or 1.0%; 3×/wk for 6 wk	Bronchoconstriction to methylcholine	N/C in airway response to MCh by FA or FA with passive sensitization, but induced by FA with active sensitization	**Not Informative [formalin; high, unknown levels; short periodicity; exposure route]**
		Sensitization: intradermal anti-OVA serum on day 38 (passive) or i.p. 2 mg OVA on day 3 (active) with boost i.p. 10 mg OVA Day 24 Challenge: 1 mg/mL nebulized OVA 15 min after last FA exposure on day 45			
(Lee et al., 1984)	Male English guinea pigs (n=4)	Formalin: 7.38 or 12.3 mg/m^3 for 5 d FA challenge with 2.46 or 4.9 mg/m^3 for 1 or 4 hr, respectively on Days 7, 22, and 29 Respiratory rate change from prechallenge baseline		N/C in pulmonary sensitivity (either immediate or delayed-onset) to formaldehyde challenge Note: 2/4 animals exhibited dermal sensitivity (likely contact-mediated) to topical FA; 12.3 mg/m^3 caused 40−50% respiratory rate decrease for ≥5 hr (later time points NR)	**Not Informative [formalin; small sample size; high exposure levels; no comparison to controls with no prior formaldehyde exposure (unclear if this, by itself, caused effects); unclear reporting]**
(Lino-Dos-Santos-Franco et al., 2013a)	Female Wistar rats (n=5)	Formalin 1% or methanol vehicle for 3 d (90 min/d), ± ovariectomy	Lung oxidative stress, microvascular leakage and mast cell degranulation; ex vivo tracheal reactivity	1 d after OVA challenge: FA/OVA versus OVA alone: Reduced MPO and vascular permeability; decreased mast cell degranulation Decreased tracheal reactivity	**Not Informative [formalin (MeOH controls), naïve not chamber exposed; high. unquantified levels, FA alone untested; small sample size]**
		Sensitization: After FA, s.c. 10 µg OVA, with s.c. boost 7 d later Challenge: After 7 d, 1% OVA aerosol for 15 min			
(Lino-Dos-Santos-Franco et al., 2011a)	Female Wistar rats (n=5)	Formalin 1% or naïve for 3 d (90 min/d), with or without ovariectomy	Ex vivo trachea response	N/C in ex vivo tracheal response to methacholine	**Not Informative [formalin, naïve not chamber exposed; ex vivo; high, unquantified levels, FA alone untested; small sample]**
(Lino dos Santos	Male Wistar (n=5−6)	Formalin 1% or methanol vehicle for 4 d (30, 60, or 90 min/d)	Ex vivo airway responsivity	Decreased ex vivo bronchial, but not tracheal, response to methacholine	**Not Informative [formalin (MeOH controls); naïve not chamber exposed; high,**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
Franco et al., 2006)				Note: number of cells recovered in BAL was significantly reduced by capsaicin (depletes neuropeptides from sensory nerve endings), but bronchial hyporesponsiveness not altered; conversely L-NAME (inhibits NO synthase) did not affect BAL cells, but did restore bronchial responsiveness; administration of 48/80 to deplete mast cells blunted FA-induced effects on both BAL cell counts and bronchial response	**unquantified levels, comparisons to naïve rats rather than MeOH controls; small sample size]** NOTE: if a relevant MOA is identified from more informative studies, pharmacological intervention endpoints might be reconsidered
(Lino-Dos-Santos-Franco et al., 2013b)	Male Wistar rats (n=5–8)	Formalin 1% or naive for 3 d (90 min/d), with or without subsequent OVA Sensitization: after FA inhalation, s.c. 10 µg OVA with same boost 7 d later Challenge: after 1 wk, 1% OVA aerosol for 15 min	Ex vivo bronchial response to MCh	Prior FA exposure reduced OVA-induced ex vivo bronchial hyperresponsiveness Note: N/C in respiratory resistance or elastance with FA alone	**Not Informative [formalin; naïve not chamber exposed; high, unquantified levels; short duration and periodicity; comparisons did not include all relevant controls (e.g., FA alone; air alone); small sample size]**

Table A-69. Serum (primarily) antibody responses

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
Observational Epidemiology Studies					
(Wantke et al., 1996a)	Human children in schools (n=62) vs. control (n=19)	Particleboard schools: 0.053, 0.085, or 0.092 mg/m^3 (n=18, 22, 22); brick schools: 0.036, 0.028, or 0.032 mg/m^3 (n=18, 22, 22); unclear duration (<2.5 yr)	Serum FA-specific IgE	Before switching schools, 40% of students had elevated FA-specific IgE, which significantly decreased 3 mos after switch to low-FA schools (p <0.002) Note: while symptoms correlated to FA levels, FA-specific IgE did not	**High or Medium Confidence [no blinding, but not clearly an issue]** Note: Natural experiment (pre- and postschool switch) with limited exposure contrast and assays
(Kim et al., 1999)	Human medical students (n=167)	3.74 ± 3.48 mg/m^3 for up to 4 yrs of school (periodicity NR)	Serum FA-specific IgG and IgE (antibodies to	14 (8.4%) students had FA-specific IgG, which was not related to duration of	**High or Medium Confidence** Note: Limited assays

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
	and nonexposed controls (n=67)		FA-human serum albumin conjugate)	schooling (No relationship to symptoms) N/C in FA-specific IgE	
(Aydın et al., 2013)	Human male fiberboard workers	0.25±0.074 mg/m^3 (average 7.3 yr employed; n=46) vs. nonexposed controls	Serum Antibodies	Decreased IgG and IgM N/C in IgA	**High or Medium Confidence**
(Wantke et al., 1996b)	Human medical students (n=45)	0.153± 0.062 mg/m^3 for 4 wk (Total: 17 d; 51 hr); phenol co-exposure	Serum FA-specific IgE Total IgE	N/C in FA-specific IgE; N/C in total IgE	**Low Confidence [37% participation; phenol co-exposure; limited periodicity]** Note: limited assays
(Wantke et al., 2000)	Human medical students (n=27); 23 controls	0.265± 0.07 mg/m^3 for 5 or 10 wks (intermittent—not specified, but assumed ≈3 hr/d)	Serum Antibodies and FA-specific Antibodies	After 5 wk: N/C FA-IgE or Total IgE After 10 wk: 4/27 students developed IgE against FA-albumin, but 0/23 developed IgG; N/C in Total IgE	**Low Confidence [no reporting of % participation or population demographics; limited, unclear periodicity; phenol co-exposure]** Note: 1 of 4 positive was a smoker (4 smokers in study); limited assays
(Erdei et al., 2003)	Human (sex NR) symptomatic students (9–11 yo w/ respiratory issues) (n=176)	0.006–0.057 mg/m^3 (average= 0.018 mg/m^3); duration unknown [co-exposure: NO_2, benzene, toluene, xylene, and dust mite allergen]	Serum Antibodies	N/C total IgG, IgA, IgM, or IgE (data NR) Increased airway pathogen bacteria-specific IgG (not IgA or IgM) with FA	**Low Confidence [comparisons to "normal" range rather than to control group; co-exposure; limited reporting]** Note: symptomatic only; authors hypothesized increased bacterial-specific IgG may represent increased B cell response (maybe more infections)
(Zhou et al., 2005)	Human anatomy students (n=8)	0.74 ±0.11 mg/m^3 (4-wk course—intermittent)	Serum FA-specific IgE antibodies	No students had FA-specific IgE after exposure	**Low Confidence [small sample (n=8); limited, unclear periodicity; reporting as yes/no rather than analytical results, and no clear comparison to preexposure]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Ohmichi et al., 2006)	Human anatomy students (n=8 measured for FA; n=6 for FA-specific IgE)	0.41-1.81 mg/m^3 (20 laboratory sessions over 10 wks; laboratory sessions ranged from 1.1−10 hrs, averaging 3hr)	Serum IgE and FA-specific IgE (threshold of 0.34 UA/mL)	No significant changes in IgE, and no positive result for FA-specific IgE (data presented was highly variable), as compared to measure 90 min before 1st session of laboratory course	**Low Confidence [small sample (n=6–8); limited and variable periodicity]**
(Thrasher et al., 1987)	Human sympto-matic exposed subjects, controls (n=8/ group)	Exposed (mobile home measures): 0.086−0.68 mg/m^3 (residency ≈6−7 yr); nonexposed: not measured (authors assume: <0.037)	Serum FA-specific IgG and IgE	No detection of FA-specific IgE Increased FA-specific IgG in all 8 exposed subjects, but only in 1/8 controls (had PD)	**Low Confidence [small sample; symptomatic vs. nonsymptomatic comparison; reporting limitations]**
(Dykewicz et al., 1991)	Human medical volunteers (n=55; 31 F, 24 M)	Generally, 0.25−0.79 mg/m^3 (1 subject up to 13.5 mg/m^3); duration 4.53 ± 1.09 yr	Serum FA-specific IgG and IgE	N/C in incidence of FA-HSA- specific IgG or IgE (3 subjects had FA-specific IgG and IgE, and 2 more had FA-specific IgG only)	**Low Confidence [periodicity unspecified; unclear exposure comparison- control levels NR and variable range in exposed]**
(Thrasher et al., 1990)	Human various exposed groups of patients, and asymptomatic controls	"controls"—chiropractic students (n=28): assumed ≥ 0.53 mg/m^3 for 28 wk (13 hr/wk); mobile home residents (n=19): 0.05−0.62 mg/m^3 for 2−7 yr; office workers (n=21): assumed 0.012−0.95 mg/m^3, duration N/R; occupational (n=8): levels/ duration N/R; removed from exposure for ≥1 yr: 0.17−1.0 mg/m^3	Serum FA-specific IgG, IgM, and IgE Blood autoantibodies	Proportion of pooled titers (IgG, IgM, and IgE) of FA-specific antibodies (i.e. % at ≥ 1:8) was greater in all patient groups than in controls (Note: most apparent for IgG, but others also appear elevated; FA-specific IgE was not found in any of the patients "removed" from exposure) Mobile home residents and office workers had increased autoantibodies vs. controls (i.e., antismooth muscle or antiparietal cell)	**Low Confidence [controls not unexposed; patients to nonpatients comparisons questionable]** Note: authors argue only real difference between asymptomatic control students and patients is one of duration of exposure
(Górski and Krakowiak, 1991)	Human textile and shoe makers (n=367)	Not exceeding 0.5 mg/m^3 (duration at least 1 yr (average ≈12 yrs)	Serum FA-specific IgE Antibodies	No FA-specific IgE in patients tested (seems to be testing in a small subset of all subjects)	**Low Confidence [incomplete and confusing methods and results; comparisons unclear]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Palczynski et al., 1999)	Human apartment house residents (n=465 total, ≈40% children)	3 categories of exposure: <0.025, 0.025–0.05, and >0.0501 mg/m^3; duration unclear, periodicity assumed to be constant	Total serum IgE Note: N=1–2 at high HCHO levels; N=27–38 at mid, low levels Serum antibodies to FA	Total IgE was not changed at 0.025–0.5 as compared to <0.025 in children or adults (n size at >0.05 was too small to compare); No FA-specific antibodies were detected (details NR); note: children exposed to 0.025–0.05 mg/m^3 and tobacco smoke had elevated IgE	**Low Confidence: IgE [small sample size; subsampling for IgE not reported; minimal exposure differential; results not stratified by sex or smoking status]** **Not Informative: FA antibodies [methods NR; data NR]**
(Madison et al., 1991)	Human residents, spill-exposed (n= 41) or unexposed controls (n=29)	Formaldehyde (PFA): >2.46 mg/m^3 for first 48 hr, then average dropped to 0.028 mg/m^3, but urea and methylamines unmeasured/not corrected	FA-specific serum antibodies and autoantibodies	N/C in FA-specific IgE Increased FA-specific IgM and IgG Increased odds ratio of having 1+ autoantibodies (although higher, no sig. increase in any one auto-antibody)	**Not Informative [mixture exposure; co-exposures not corrected for; FA in controls unmeasured]**
(Grammer et al., 1990)	Human workers (Boeing; n=37); details N/R	0.0037–0.090 mg/m^3 (not stratified by exposure; all exposed; duration N/R)	Serum FA-specific IgG and IgE	0/37 had FA-specific IgG 5/37 had elevated IgE (vs. control sera) that was not specific to FA-HSA or HSA	**Not Informative [details on population N/R; details on exposure NR; no specific comparison to FA levels]**
Controlled-Exposure Studies in Animals, Animal Cells, or Immortalized Human Cells					
(Fujimaki et al., 2004b)	Female C3H mice (n=5–6 per group)	PFA 0, 0.098, 0.49, or 2.46 mg/m^3; 12 wks (16 hr/d, 5 d/wk) Sensitization: i.p. 10 µg OVA prior to FA exposure; aerosol OVA boost for 6 min on wks 3, 6, 9, and 11	Serum Antibodies and Antibodies to Antigen	No change in anti-OVA IgE (variable) or IgG_{2a} or Total IgE Decreased anti-OVA IgG_1 (at 0.49 mg/m^3 only) and IgG_3 (at 0.098–0.49 mg/m^3) Body weight decreased 20% at 0.49 mg/m^3	**High or Medium Confidence [slightly small sample size]**
(Riedel et al., 1996)	Female Dunkin-Hartley guinea pigs (n=12)	Formaldehyde (bottled pressurized gas) 0, 0.13, 0.31 mg/m^3 for 5 d (8 hr/d); Sensitization: 0.5% inhaled OVA; OVA boost at 2 wk Challenge: 1% inhaled OVA 1 wk later	Serum OVA-specific IgG1	Increased OVA-specific IgG1 by 0.31 mg/m^3	**High or Medium Confidence [no comparison group with FA without OVA]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Sapmaz et al., 2015)	Male SD rats (n=5–7)	PFA 0, 6.15, 12.3 mg/m^3; 4 wks (8 hr/d, 5 d/wk)	Serum Antibodies	Increased IgA, IgM, and complement 3 Decreased IgG	**High or Medium Confidence [slightly small sample size; high formaldehyde levels]**
(Tarkowski and Gorski, 1995)	Female Balb/c mice (n=4/ group)	Formalin (assumed; test article N/R) 0 or 2 mg/m^3 for 10 d (6 hr/d) or 7 wk (6 hr/d, 1 d/wk) Sensitization: intranasal 25 µg OVA 1x/wk for 7 wk OR i.p. 1 µg OVA 1×/wk for 4 wk	Serum OVA-specific IgE	Increased OVA-specific IgE in mice exposed for 10 d, but not in those exposed 1x/ wk, as compared to controls Specific to nasal tissue, as OVA sensitization via i.p. injection caused N/C	**Low Confidence [formalin; small sample size]** Note: pinpoints issue of importance and interpretability of different sensitization methods
(Wu et al., 2013)	Male Balb/c mice (n=8/group)	Formalin 0, 3 mg/m^3 for 4 wk (6 hr/d, 5 d/wk) Sensitization: s.c. 80 µg OVA on days 10, 18, and 25 Challenge: 1% OVA aerosol 30min/d on day 29-35	Serum antibodies	FA alone increased total IgE, but not OVA-IgG or OVA-IgE; FA+OVA increased IgE compared to OVA alone, but did not further elevate OVA-IgG or OVA-IgE (slight, NS increases) compared to OVA TRPA1 and TRPV1 antagonists reduced FA+OVA-induced serum antibodies	**Low Confidence [formalin; pharmacological interventions did not include effects of FA alone]**
(Kim et al., 2013b)	Female NC/Nga (atopic-prone) mice (n=5–7/group)	Formalin (assumed; test article NR) 0, 0.25, 1.23 mg/m^3 for 4 wk (6 hr/d, 5 d/wk) Sensitization: topical house dust mite (HDM; ear) stimulation (25 mg Df ointment) 1×/wk for 4 wk	Plasma Antibodies and Antigen-specific Abs	Plasma IgG1 increased by FA alone (0.25 mg/m^3 only), but N/C in total IgE or IgG2a FA exacerbates HDM-induced IgE (≥0.25 mg/m^3) and IgG2a (0.25 mg/m^3 only), but not IgG1 HDM-specific IgE not changed	**Low Confidence [formalin; small sample size]** Note: multiple supplementary files; HDM-specific IgE data NR
(Gu et al., 2008)	Female Balb/c mice (n=5–6/ group)	Formalin (assumed; test article NR) 0.12 or 0.98 mg/m^3 for 5 wk (24 hr/d, 5 d/wk) Sensitization: i.p. 10 mg OVA on day 0 and 7 pre-FA	Serum Antibodies and OVA-specific Antibodies	N/C in total serum IgG or IgE Increased OVA-specific IgE in allergen primed host, only at 5 wks (not ≤ 4 wk) and only at 0.98 mg/m^3; N/C in OVA-IgG	**Low Confidence [formalin; small sample size]**
(Jung et al., 2007)	Female C57BL/6 mice (n≥5/ group)	Formalin (assumed; test article NR) 0, 6.15, 12.3 mg/m^3 for 2 wk (6 hr/d, 5 d/wk)	Serum Antibodies	Increased Total IgG1, IgG3, IgA, and IgE Decreased Total IgG2a and 2b; N/C IgM Note: body wt decreased ≈10%	**Low Confidence [formalin; high exposure levels; small sample size]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Holmstrom et al., 1989a)	Female SD rats (n=8−9 treated rats; n=6 control)	Formalin (assumed; test article NR) 15.5 ± 2.3 mg/m^3 for 22 mos (6 hr/d, 5 d/wk); all rats vaccinated: anti-tetanus and Pneumovax	Serum antibody response to vaccination	N/C in IgM response to vaccine-related antigens Variable increases in IgG against specific antigens were not statistically significant (Note: IgE not measured)	**Low Confidence [formalin; excessively high exposure level; no unvaccinated comparison group]** Note: authors indicate B cell function unchanged
(Lee et al., 1984)	Male English guinea pigs (n=4)	Formalin: 7.38 or 12.3 mg/m^3 for 5 d, with FA challenge with 2.46 or 4.9 mg/m^3 for 1 or 4 hr, respectively	Serum antibody to formaldehyde (isotype not specified) measured 9 or 17 d (i.e., days 14 or 22) after exposure	N/C antibody response to 2.46 or 4.9 mg/m^3 (data NR) Note: 2/4 animals exhibited dermal sensitivity (likely contact-mediated) to topical FA	**Low Confidence [formalin; small sample size; high exposure levels]** Note: although there was no comparison to controls with no prior formaldehyde exposure, this is not expected to affect this measure
(Sadakane et al., 2002)	Male ICR mice (n=9 or 18)	Formalin 0.5% for 4 wk (15 min/wk) ± sensitization of house dust mite allergen (Der f)	Blood Der f-specific IgG1 and IgE	N/C in Der f-specific IgG1 or IgE (latter appears to have been lower than detection limit)	**Low Confidence [formalin; high, unknown exposure levels; short periodicity]**
		Sensitization: i.p. with 3 mg/mL Der f (house dust mite allergen) prior to FA Challenge: intratracheal 10 µg Der f 3 hr after last exposure (note: measures 3 d later)			
(Kita et al., 2003)	Male Hartley guinea pigs (n=5-7/group)	Nasal Instillation of saline or Formalin 0.1 or 1.0%; 3×/wk for 6 wk	PCA reaction of naïve animals to injected serum of exposed animals	Increased anti-OVA IgG at ≥0.1% FA (at 4 hr, but not 7 d after OVA challenge) in naïve animals injected with serum	**Not Informative [exposure route; formalin; high, unknown exposure levels; short periodicity; small sample size (for some endpoints/ groups)]**
		Sensitization: i.p. anti-OVA serum on after 5 wk FA (passive) or i.p. 2 mg OVA on day 3 (active) prior to FA exposure with boost i.p. 10 mg OVA day 24 Challenge: 1 mg/mL nebulized OVA 15 min after last FA exposure on day 45			
(Lino dos Santos Franco et al., 2009)	Male Wistar rats (n=5)	Formalin 0, 1% for 3 d (90 min/ d)	Skin Antibodies	N/C in skin IgE	**Not Informative [formalin; unquantified high exposure levels; small sample size; short duration and periodicity]**
		Sensitization: immediately post-FA, i.p. 10 µg OVA; boost 1 wk later with s.c. injection			

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
		Challenge: 1 wk later with aerosolized OVA			Note: unclear endpoint relevance
(Lino-Dos-Santos-Franco et al., 2013a)	Female Wistar rats (n=5)	Formalin 1% or methanol vehicle for 3 d (90min/d), ± ovariectomy	Skin IgE	1 d after OVA challenge: FA/OVA vs. OVA alone: N/C in cutaneous OVA-specific IgE	**Not Informative [formalin (MeOH controls); unquantified high exposure levels; small sample size; short duration and periodicity; naïve not chamber exposed]** Note: unclear endpoint relevance
		Sensitization: After FA, s.c. 10 µg OVA, with s.c. boost 7 d later Challenge: After 7 d, 1% OVA aerosol for 15 min			

Table A-70. Serum markers of immune response (other than antibodies), inflammation, or oxidative stress

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
Observational Epidemiology Studies					
(Aydın et al., 2013)	Human male fiberboard workers	0.25 ± 0.074 mg/m^3 (average 7.3 yr employed; n=46) vs. nonexposed controls	Serum cell counts, cytokines and related factors	N/C in # hematologic cells, WBC, RBC, Hb, neutrophils, or monocytes; N/C in helper T, suppressor T, or B lymphocytes Increased % of lymphocytes, and numbers and % of T cell (CD3+) and NK cell (CD56+) Increased TNFα, but N/C in Complement 3 or 4; TNFα increased more significantly in those not using protective measures	**High or Medium Confidence** Note: annex reviews immune data
(Bassig et al., 2016) (same cohort as (Zhang et al., 2010)	Human melamine workers (n=43) or n=51 age- and sex-matched unexposed from different factories in the same region of China	1.6 mg/m^3 (10% and 90% = 0.74 and 3.08 mg/m^3); unclear exposure duration (sampling over a 3-wk period)	Serum cell counts and soluble markers	Decreased total WBC, Granulocytes, Monocytes, Platelets, and Lymphocytes Decreased CD8+ cells (CD8 effector memory cells most affected) and NK cells N/C in Monocytes, CD4+ cells, CD4/CD8 ratio, or B cells; N/C in soluble CD27 or CD30	**High or Medium Confidence**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Costa et al., 2013)	Human pathology anatomists (n=35) or administrative controls (n=35)	0.44 ± 0.037 mg/m^3 (as high as 0.85 mg/m^3 in peaks); duration of employment ≥ 1yr	Serum lymphocyte subtypes	Decreased B cells (% CD19+) in exposed N/C in T cells or NK cells in exposed Within the exposed workers: FA exposure level correlated with Increased % T cells (CD3+) and % T helper cells (CD4+), and decreased % NK cells	**High or Medium Confidence** Note: authors suggest immunosuppression
(Costa et al., 2019)	Human anatomy-pathology lab workers (n=85) or administrative controls (n=87)	8 hr TWA=0.47 ± 0.037 mg/m^3 (range=0.098–1.71 mg/m^3; as high as 3.94 mg/m^3 in peaks); duration of employment average ≈12 yr	Serum lymphocyte subtypes	Increased Cytotoxic (CD8+) T cells and NK cells; Decreased B cells and CD4/CD8 ratio; N/C in total T cells or Helper (CD4+) T cells	**High or Medium Confidence** Note: authors suggest immunostimulation
(Zhang et al., 2010)	Human formaldehyde melamine workers	51 Controls: <0.037 mg/m^3; 43 Exposed: 1.8 (0.42–6.9) mg/m^3; Duration at least 3 mos (41/43 exposed > 1 yr)	Serum immune markers	22/38 immune/inflammation markers that were detectable were decreased Stringent FDR cutoff (10%): significantly decreased CXCL11 and CCL17 (both ≈25%) FDR at 20%: significantly decreased CRP, TRAIL, SAP, IL-10, sCD40L, and Insulin N/C in TNF-a; other markers below LOD	**High or Medium Confidence** [Note: the strongest correlation of marker changes was with monocyte levels (p = 0.05), but overall the results suggest that cell counts do not explain the marker changes]
(Zhang et al., 2010)	Human formaldehyde melamine workers	51 Controls: <0.037 mg/m^3; 43 Exposed: 1.57 (0.77–6.9) mg/m^3; Duration at least 3 mos (41/43 exposed > 1 yr)	Serum cell counts Proliferation of serum hematopoietic progenitor cells	Decreased WBC, lymphocytes, granulocytes, platelets, and RBC Increased mean corpuscular volume N/C in monocytes, hemoglobin Decreased colony formation in cultured hematopoietic progenitors from subjects	**High or Medium Confidence [one ex vivo endpoint: possible influence of culturing- still expected to be due to exposure, but could involve in vitro amplification of phenomena]**
(Jia et al., 2014)	Human plywood workers (n=118) and controls (n=79)	[High] workers: 0.77 (0.44–1.88) mg/m^3 (n=70); [Low] workers: 0.18 (0.086–0.23) mg/m^3	Serum lymphocyte subtypes and cytokines	Dose-dependent increased % CD19+ B cells at ≥ 0.18 mg/m^3; increased CD56+ NK cells at 0.18 mg/m^3 only N/C in %CD3+, CD4+ or CD8+ T cells	**High or Medium Confidence**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
		(n=48); duration ≥6 mos; controls <0.01 mg/m^3		Increased IL-10 and decreased IL-8 at ≥ 0.18 mg/m^3; Increased IL-4 and decreased IFNγ at 0.77 mg/m^3	
(Hosgood et al., 2013) Note: Same cohort as (Zhang et al., 2010)	Human formaldehyde melamine workers	51 Controls: 0.032 (0.01–0.032) mg/m^3; 43 Exposed: 1.57 (0.77−3.09) mg/m^3; Duration at least 3 mos (41/43 exposed >1 yr)	Serum counts and analyses of lymphocyte subsets	Decreased lymphocytes, NK cells, T cells, and CD8+ T cells N/C in B cells, or CD4+ T cells (overall; note: CD4+/FoxP3+ decreased) T cells subset analyses showed decreased CD8+ effector T cells and regulatory T cells	**High or Medium Confidence** Note: Authors hypothesized decreased effector T cells (which circulate to inflamed tissues) may reflect decreased response to antigenic-related inflammation, and decreased regulatory cells as decreased immunosuppression (which may lead to autoimmunity)
(Ye et al., 2005)	Human students (n=23), waiters (n= 16), or FA manufacturers (n=18)	[High] Manufacturers: 0.98 5± 0.286 mg/m^3 (8.5 yr, 8 hr/d; 1.69 maximum); [Low] waiters: 0.107 ± 0.067 mg/m^3 (12 wk, 5 hr/d); Controls: 0.015 mg/m^3	Blood lymphocyte subset analysis	N/C in waiters exposed to low levels Increased % B cells and ratio of T helper to T cytotoxic T cells (CD4/CD8 ratio), and decreased total T cells and CD8+ T cells in workers exposed to high levels	**High or Medium Confidence [data not adjusted for age or gender]**
(Bono et al., 2010)	Human pathologists (n=44) and controls (n=32)	Controls: 0.028 ± 0.0025 mg/m^3; Pathologists: 0.032 ± 0.006 or 0.21 ± 0.047 mg/m^3 (in "reduction room"); duration unclear	Serum lymphocyte ROS (MDA-dG adducts)	Increased MDA-dG at > 0.066 mg/m^3; N/C in MDA-dG at <0.022 mg/m^3 or 0.023−0.066 mg/m^3 (significant association with air-FA levels)	**High or Medium Confidence (unknown duration)**
(Romanazzi et al., 2013)	Human Laminate workers (males, yrs employed NR)	0.21 ± 0.10 mg/m^3 exposed (n=51); 0.04 ± 0.02 mg/m^3 nonexposed (n=54)	15-F_{2t} Isoprostanes in urine (also measured cotinine and smoking)	Smoking and air-formaldehyde exposure were independently associated with increased IsoP	**High or Medium Confidence - indirect [accuracy of single measure questionable]** Note: serum and urine isoprostanes are correlated (Rodrigo et al., 2007); thus, this finding is indirect for serum ROS

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Lyapina et al., 2004)	Human workers with carbamide-FA glue (n=29)	Exposed workers: 0.87 ± 0.39 mg/m^3 (n=21 nonexposed); duration mean: 12.7 ± 9.6 years	Blood neutrophil oxidative burst Routine hematology Assessment of chronic URT inflammation	Significant decreases in neutrophil function/ oxidative burst were only detected when comparing the 12 workers with evidence of URT inflammation (N/C across full groups) Decreased erythrocyte count and hematocrit levels correlated with duration of exposure (no other changes)	**High or Medium Confidence [mixture exposure]** Note: Authors hypothesized that decreases in erythrocyte and hematocrit counts might indicate FA toxicity on bone marrow hematopoiesis
(Jakab et al., 2010)	Human female pathologists or controls (n=37)	0.9 mg/m^3 (8 hr-TWA exposure); mean duration >17 yrs; slightly more (not significant) smokers and drinkers in exposed	Serum lymphocyte parameters: CD71 in fresh cells; apoptosis/ proliferation in cells cultured with PHA	N/C in T cell activation marker, CD71 Exposure to FA alone increased apoptosis and 1 out of 3 measures of cell proliferation in PBLs; N/C % in S phase	**High or Medium Confidence - CD71 [limited precision of exposure assessment - sampling 1–3 yrs from study]** **Low Confidence -other measures [ex vivo; limited exposure assess]**
(Bellisario et al., 2016)	Human nurses (Italian females, yrs employed NR)	0.034 ± 0.038 mg/m^3 using formalin (n=64); 0.015 ± 0.005 mg/m^3 not using formalin (n=30), but noting that they did receive some exposure; 8-hr workshift measures on 2 separate days	15-F_{2t} Isoprostanes and malondialdehyde in urine, normalized to creatinine (also measured cotinine)	Smoking and air-formaldehyde exposure were independently (positively) associated with increased oxidative stress biomarkers by pairwise comparisons and regression (note: in nurses who used vacuum sealing techniques, which reduce formaldehyde exposure, also exhibited reduced biomarkers).	**Low Confidence - indirect [accuracy of single measure questionable]; small exposure differential; formalin test article** Note: serum and urine isoprostanes are correlated (Rodrigo et al., 2007); thus, this finding is indirect for serum ROS
(Erdei et al., 2003)	Human (sex NR) symptomatic students (9–11 yo w/ respiratory issues) (n=176)	0.006–0.057 mg/m^3 (average= 0.018); duration unknown [co-exposure: NO_2, benzene, toluene, xylene, and dust mite allergen]	Serum Cell Counts	Increased serum monocyte counts by linear regression; N/C in RBCs, WBCs, platelets, lymphocytes, neutrophils (mostly), or eosinophils (all data NR)	**Low Confidence [comparisons to "normal" range rather than to control group; co-exposure; limited reporting]** Note: symptomatic only
(Kuo et al., 1997)	Human dialysis nurses (n=51) or ward nurses controls (n=71)	Personal sampling ranged from 0.018–0.11 mg/m^3; area sampling was as high as 3.44 mg/m^3 (duration	Blood cell counts	WBC decreased in 2nd blood test (1 year after the first test at study onset-N/C): associated with FA concentration and symptoms, but not work duration (correlated, but N/S)	**Low Confidence [not clear that controls are appropriately unexposed nor what co-exposures exist]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
		average= 3 yr; ≈1/3 employed <1 yr and ≈40% > 3 yr); control area levels N/R		N/C RBC, Ht, MCV, MCH, MCHC, Plt, neutrophil, lymphocyte, monocyte, eosinophil, or basophils	(Note: 2nd blood test, presumably, would involve an extra 1 yr of exposure duration)
(Thrasher et al., 1987)	Human sympto-matic exposed subjects, controls (n=8/ group)	Exposed (mobile home measures): 0.086–0.68 mg/m^3 (residency ≈6−7 yr); nonexposed: not measured (authors assume: <0.037)	Serum cell counts Ex vivo T and B cell blastogenesis (PHA or PWM stimulation)	T cell number decreased; B cell counts were not significantly changed T cell blastogenesis with PHA (not PWM: p>0.05, authors call significant) impaired	**Low Confidence [small sample; symptomatic vs. nonsymptomatic comparison; questionable reporting]**
(Thrasher et al., 1990)	Human various exposed groups of patients, and asymptomatic controls	"controls"- chiropractic students (n=28): assumed ≥ 0.53 mg/m^3 for 28 wk (13 hr/wk); mobile home residents (n=19): 0.062–0.62 mg/m^3 for 2−7 yr; office workers (n=21): assumed 0.012–0.95 mg/m^3, duration N/R; occupational (n=8): levels/ duration N/R; removed from exposure for ≥ 1 yr: 0.17−1.0 mg/m^3	Blood cell counts	Decreased WBCs in office workers; N/C in all T cells, T helper or T suppressor cells, or T cell H/S ratio Ta1+ lymphocytes (antigenic stimulation) elevated in all exposed patient groups B cells increased in office workers and removed patients IL2R+ lymphocytes increased in mobile home residents and removed patients	**Low Confidence [limited exposure contrast- authors suggest the only real difference between asymptomatic control students and patients is one of duration of exposure; patients to nonpatients comparisons questionable]**
(Ying et al., 1999)	Human anatomy students (n=23)	0.508 ± 0.3 mg/m^3 for 8 wks (3 hr/d, 3 d/ wk); in dormitories: 0.012 ± 0.003	Serum lymphocyte subsets Ex vivo lymphocyte proliferation (culture lymphoblast counts)	After exposure compared to before exposure: Increased % B cells (CD19), decreased Total T cells (CD3), T helper (CD4) and T cyto. (CD8) cells; N/C in ex vivo lymphocyte proliferation rate	**Low Confidence [limited periodicity; some experiments ex vivo]** Note: internally controlled
(Madison et al., 1991)	Human residents, spill-exposed (n= 41) or unexposed controls (n=29)	Formaldehyde (PFA): >2.46 mg/m^3 for first 48 hr, then average dropped to 0.028 mg/m^3, but urea and methylamines not	Serum cell counts	N/C in WBC, lymphocyte, CD8, CD8/CD4 ratio, CD19, or CD25 cells Decreased % CD5+ and % CD4+, although total counts of these were unchanged Increased CD26+ counts and %	**Not Informative [mixture exposure; co-exposures not corrected for; FA in controls unmeasured]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
		measured or corrected for			
(Vargová et al., 1992)	Human Woodworkers (Czechoslovakia)	Formaldehyde 0.55–10.36 mg/m^3 and other, unquantified exposures	Serum IgG, IgA, IgM, IgE Complement and other factors Lymphocyte proliferation	Increased lymphocyte proliferation to concanavalin A and decreased proliferation to phytohaemaglutinin "no significant differences in natural cellular and specific humoral immunity"	**Not Informative [mixture exposure; co-exposures not corrected for; FA in controls unmeasured; no description of recruitment or how referents were matched- reporting limited]**
(Zhang et al., 2010)	Human formaldehyde melamine workers	51 Controls: <0.037 mg/m^3; 43 Exposed: 1.57 (0.77–3.09) mg/m^3; Duration at least 3 mos (41/43 exposed >1 yr)	In vitro proliferation of a volunteer's cells	Decreased colony formation in cultured progenitors with in vitro FA treatment	**Not Informative [formalin treatment- assumed; single donor, in vitro; nongaseous exposure, levels relevance]**
Controlled-Exposure Studies in Humans or Primary Human Cells					
(Dietrich et al., 1996)	In vitro human leukocytes (single donor): not further described	Formalin (assumed; test article N/R) gas at 0.62 mg/m^3 for 1 hr	Heat shock protein 70 levels (Westerns)	FA, but not heat (42°C) stress, caused a significant increase in HSP70 levels	**Not Informative [formalin; in vitro; short duration; exposure level relevance unknown; sample size NR; poor reporting]**
Controlled-Exposure Studies in Animals, Animal Cells, or Immortalized Human Cells					
(Sorg et al., 2001a)	Male SD rats (n=6–9/ group)	PFA (inferred from citation) 0, 0.86, or 2.95 mg/m^3 for 20–60 min, 2 or 4 wk	Serum corticosterone	N/C with acute exposure Increased CORT at 2.95 mg/m^3 at 2 or 4 wk	**High or Medium Confidence** Note: unclear utility of endpoint for respiratory effects interpretation
(Rager et al., 2014)	Male fischer rats (n=3)	PFA 0 or 2.46 mg/m^3 for 7 d, 28 d or 28 d with 7d recovery (6 hr/d)	miRNA microarray of blood WBCs	WBCs miRNAs were changed after 7 d or 28 d or 28 d with recovery (31 or 8 or 3 transcripts); associated primarily with inflammation and immunity	**High or Medium Confidence [small sample size]** Note: unclear/indirect interpretation of endpoints
(Morgan et al., 2017)	Male B6.*Trp53*^{tm1Brd} and C3B6.129F1-*Trp53*^{tm1Brd} mice (heterozygote P53 allele); n=25/group	PFA 0, 9.23, or 18.45 mg/m^3 for 8 wks (6 hr/d, 5 d/wk) with measures at approximately 1 yr	Whole blood counts	N/C in hematological parameters, including RBC, WBC, neutropils, monocytes, eosinophils, platelets, lymphocytes, reticulocytes, hemoglobin, hematocrit, MCV, MCH, or MCHC	**High or Medium Confidence**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Dean et al., 1984)	Female B6C3F1 mice (n=10/ group)	PFA 0 or 18.5 mg/m^3 for 3 wk (6 hr/d, 5 d/wk)	Serum cell counts	N/C peripheral blood cell counts, including WBC differentials, except: Decreased number of monocytes (from 43 to 4)	**Low Confidence [excessively high levels: 60-70% RB inferred at these levels]** Note: monocyte decrease speculated as peripheral response to nasal inflammation and healing
(Aydin et al., 2014)	Male SD rats (n=6/ group)	Test article unclear, but appears to be formalin in this experiment at 0, 6.48 (low), 12.3 (moderate), or 18.7 mg/m^3 for 4 wk (8 hr/d, 5 d/wk)	Serum total antioxidant and total oxidant levels (TAS and TOS; kit uses vitamin E and H_2O_2 as reference, respectively Serum oxidative stress index (OSI: TOS/TAS) Serum irisin (hormone may regulate obesity)	Increased TOS, and decreased TAS and irisin, at ≥ 12.3 mg/m^3 formaldehyde Increased OSI at ≥6.48 mg/m^3 Note: serum biochemical parameters (e.g., cholesterol) are not included here, but were unchanged. Carnosine supplementation reduced changes.	**Low Confidence [formalin; high levels]**
(Zhang et al., 2013)	Male Balb/c mice (n=9)	Formalin 0, 0.5, or 3 mg/m^3 for 2 wk (8 hr/d, 5 d/wk)	Serum cell counts	D/D Decreased serum WBC, RBC, and lymphocytes, and increased platelets, at ≥0.5 FA; decreased intermediate cells at 0.5 FA; N/C in neutrophils	**Low Confidence [formalin]**
(Ye et al., 2013)	Male Balb/c mice (n≥9/ group/ endpoint)	Formalin 0, 0.5, 1, or 3 mg/m^3 for 7 d (8 hr/d)	ROS (dichlorohydro-flourescein and MDA) blood mononuclear cells (PBMC)	Dose-dependent decrease in GSH levels in PBMC at ≥1 Dose-dependent increase in DCFH and MDA in PBMC at 3 Co-administered GSH attenuated effects	**Low Confidence [formalin]**
(Im et al., 2006)	Male SD rats (n=10)	Formalin (assumed; test article not specified) 0, 6.15, 12.3 mg/m^3 for 2 wks (6 hr/d; 5 d/wk)	Plasma ROS, cytokines, and proteomic analysis	Increased MDA & protein carbonyls at 12.3 mg/m^3 (note: similar increases in liver) D/D Increased IL-4 and decreased IFNγ Other protein changes (e.g, increased GSTs and ApoE; decreased heme	**Low Confidence [formalin; high levels]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
				oxygenase, fibrinogen, ApoA1, SNAP-25	
(Matsuoka et al., 2010)	Male ICR mice (n≥ 7)	Formalin at 0.12 mg/m^3 for up to 24 hr; also, a single experiment at 3.69 mg/m^3 for 24 hr	Plasma ROS (8OHdG) and NO (nitrates/ nitrites); NO response to LPS injection: 3.69 mg/m^3	Increased plasma ROS at 0.12 mg/m^3 for ≥8 hr and NO at 24 hr Increased plasma SOD activity at 3.69 mg/m^3; N/C in plasma IL-6 at 0.12 mg/m^3 Decreased NO_3 with LPS stimulation	**Low Confidence [formalin; short duration]** NOTE: ACUTE
(Sandikci et al., 2007b)	SD rats (n=6/ group) at GD1 [I], PND1 [II], PND28 [III] or adults [IV]	Formalin (assumed: test article NR): 0 or 7.38 mg/m^3 for 6 wk (8 hr/d, 7 d/wk)	Blood T lymphocyte counts	Increased blood T lymphocytes (ANAE+ as marker) in all groups by FA	**Low Confidence [formalin; high exposure levels; use of ANAE as T lymphocyte marker under all conditions has been debated]**
(Katsnelson et al., 2013)	Rat "white" females (n=12-15)	Formalin (assumed; test article NR) 12.8 ± 0.69 mg/m^3 for 10 wk (4 hr/d, 5 d/wk)	Blood cell counts and immune markers (other markers N/C or not inflammation)	Increased % lymphocytes and albumin; Decreased % segmented neutrophils, MDA, GSH, and lymphocyte SDH activity; some decreased serum amino acids	**Low Confidence [formalin; excessively high levels; short periodicity]**
(Yu et al., 2014b)	Male ICR mice (n=6)	Formalin 20, 40, 80 mg/m^3 for 15 d (2 hr/d)	Blood cell counts	Decreased blood WBCs and platelets at ≥ 40 mg/m^3	**Low Confidence [formalin; excessively high levels; short periodicity]**
(Brondeau et al., 1990)	Male SD rats (n=10)	Formalin (assumed; test article NR) 35.7–75 mg/m^3 for 4 hr, with or without adrenalectomy	Serum cell counts	Decreased WBCs at ≥ 52.9 mg/m^3, not at 35.7 mg/m^3; N/C in RBCs Adrenalectomized rats did not show decreased WBCs at 60.3 mg/m^3	**Low Confidence [formalin; excessively high levels; short periodicity]** NOTE: ACUTE
(Zhao et al., 2020)	Male Balb/c mice (n=3, pooled into single sample for nose and lung samples); 2 experiments by different researchers	Formalin 0, 3 mg/m^3 for 2 wks (8 hr/d, 5 d/wk)	Burst-forming unit-erythroid (BFU-E), and colony-forming unit-granulocyte macrophage (CFU-GM) colonies in nose, lung, spleen, and bone marrow	Bone marrow results: Decreased formation of CFU-GM and BFU-E in both experiment I and II	**Low Confidence [formalin; small sample size]** **Not Informative: ex vivo results**
(Wei et al., 2014)	Male C57BL/6 mice (n=6)	Methanol-free formalin at 0, 0.5, or 2 mg/kg/d	Serum cytokines for Th1, Th2, and Th17	Increased Th1-related cytokines (IFN-γ, TNF, and IL-2), TH2-related cytokines (IL-4, IL-6, and IL-10), and Th17-related	**Not Informative [levels of unknown relevance; i.p. injection]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
		for 1 wk or 1 mo (5 d/wk)		cytokine (IL-17A) at 2 mg/kg/d for 1 or 4 wks; **specific statistically significant increases only noted for 1 wk IL-2 and IL-4 levels** (note: magnitude of change was equal or greater at 1 mo and for all tested cytokines in all comparisons; in general, small decreased levels noted at 0.5 mg/kg)	Note: Kruskal-wallis test
(Ibrahim et al., 2016)	Pregnant Wistar rats (n=5 dams; 10 pups/ group for experiments; note: individual pup data for n=10 pups did not appear to account for litters)	Formalin 0.92 mg/m^3 from GDs 1−21: 1 hr/d, 5 d/wk	Blood cell counts and Myeloperoxidase activity	Increases in total cells and granulocytes (lymphocytes and monocytes were unchanged) by LPS were reduced in offspring exposed to formaldehyde, as were increases in myeloperoxidase activity	**Not Informative [formalin; short periodicity; offspring comparisons do not include FA without LPS; small sample size; did not appear to account for litter effects]** **Note: effects rescued by vitamin C**
		Randomly assigned pups all received 5 mg/kg lipopolysacharride (LPS) injections at PND 30			
(Maiellaro et al., 2014)	Pregnant Wistar rats (n=5)	Formalin 0.92 mg/m^3 from GDs1−21: 1 hr/ d, 5 d/wk	Blood cell counts	N/C in parental blood total cells, mono-cytes, lymphocytes, or granulocytes Decreased birth weight in offspring 24 hr after OVA challenge, offspring have: decreased blood total cells, mononuclear cells, neutrophils, and eosinophils	**Not Informative [formalin, short periodicity, offspring comparisons do not include FA alone; small sample size]**
		Sensitization: s.c. 10 µg OVA with sc boost after 7d Challenge: 7 d later, 1% OVA aerosol 15 min/d, 3 d			
(Kum et al., 2007b)	Female SD rats (n=6)	Formalin (assumed: test article NR): 0 or 7.38 mg/m^3 for 6 wks (8 hr/d, 7 d/wk)	Serum biochemistry (proteins and factors)	Increased serum urea, but N/C in total protein, albumin, or creatinine Note: experiments with FA + xylene not considered	**Not Informative [formalin; high levels; tests not considered relevant to inflammation or respiratory effects]**
(Ciftci et al., 2015)	Male Wistar albino rats (n=10)	Formalin i.p. injection at 9 mg/kg/d every other day for 2 wks	Serum markers for ROS, antioxidants, as well as beta amyloid and tumor protein 53 levels	Increased MDA (ROS marker) Decreased total antioxidants, TP53, and A-beta1-40 (not 1−-42)	**Not Informative [formalin; high levels of unknown relevance; i.p. injection]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Murta et al., 2016)	Male Fischer rats (n=7)	Formalin (assumed) 1%, 5%, or 10% for 5 d (3 × 20 min/d)	Blood cell counts, chemokine levels, and ROS indicators	FA increased total leukocyte, lymphocytes at 5%, and decreased platelets at 10%; N/C in other cell types; 1% caused increased catalase and other ROS indicators were observed; increased CCL2 at 10%, CCL3 at 1−5%, and CCL5 at 1%	**Not Informative [formalin; unquantified high levels; static exposure chamber; short periodicity]**
(da Silva et al., 2015)	Male Wistar rats (n=6/ group)	Formalin 1% for 3 d (90 min/d); rats exposed in static chambers 5 rats/time	Blood cell counts	FA increased total cells, monocytes, lymphocytes, and neutrophils Note: while reduced effects were reported as reduced with laser therapy, laser therapy-only controls were not used	**Not Informative [formalin; unquantified high levels; static exposure chamber and group exposure; short duration and periodicity]**
(Lino dos Santos Franco et al., 2006)	Male Wistar rats (n=5–6)	Formalin 1% or methanol vehicle for 4 d (30, 60, or 90 min/d)	Serum cell counts	Increased serum leukocytes and mononuclear cells, but not neutrophils	**Not Informative [formalin (MeOH controls); unquantified high levels; short periodicity; small sample size; presented comparisons to naïve rats rather than MeOH controls]**
(Lino-Dos-Santos-Franco et al., 2011a)	Female Wistar rats (n=5)	Formalin 1% or naïve for 3 d (90 min/d), with or without ovariectomy	Serum cell counts and factors	Increased total serum leukocytes Increased serum corticosterone	**Not Informative [formalin; impact of sham surgery NR; short periodicity and duration; unquantified high level; FA alone untested; naïve not chamber exposed; small sample size]**
(Lino dos Santos Franco et al., 2009)	Male Wistar rats (n=5)	Formalin 0, 1% for 3 d (90 min/d) Sensitization: immediately post-FA, i.p. 10 µg OVA; boost 1 wk later with s.c. injection Challenge: 1 wk later with aerosolized OVA	Serum cell counts	Increased Total serum leukocytes and mononuclear cells, not neutrophils; FA inhibited OVA-induced increases	**Not Informative [formalin; unquantified high level; small sample size; short duration and periodicity]**

Table A-71. Effects on other immune system-related tissues (e.g., bone marrow, spleen, thymus, lymph nodes, etc.)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
Controlled-Exposure Studies in Animals, Animal Cells, or Immortalized Human Cells					
(Fujimaki et al., 2004b)	Female C3H mice (n=5–6 per group)	PFA 0, 0.098, 0.49, or 2.46 mg/m^3; 12 wks Sensitization: i.p. 10 µg OVA prior to FA exposure; aerosol OVA boost for 6 min on wks 3, 6, 9, and 11	Splenic Cell counts Ex vivo splenic cells	No significant change in counts of splenic CD3 T cells, CD19 B cells, or CD4/CD8 ratio D/D Increased IFNγ with LPS stimulation of cells at 2.46 mg/m^3 D/D Increased MCP-1 at ≥ 0.49 mg/m^3 in cells of OVA-stimulated mice; N/C in IFNγ, MIP-1α or IL-5 Body weight decreased at ≥0.49 mg/m^3	**High or Medium Confidence [small sample size]: cell counts** **Low Confidence [small sample size; ex vivo]: cytokine measures**
(Rager et al., 2014)	Male Fischer rats (n=3)	PFA 0 or 2.46 mg/m^3 for 7 d, 28 d or 28 d with 7 d recovery (6 hr/d)	miRNA microarray of femur BM cells	N/C in BM miRNAs at any time	**High or Medium Confidence [small sample size]** **NOTE:** indirect interpretation of endpoints
(Ma, 2020, 7017056)	Male BALB/c mice (n=8)	Methanol-free formalin 0 or 2 mg/m^3 for 8 wks (8 hr/d, 7 d/w)	T cells in the spleen (mature) and thymus (immature)	Spleen: Decreased CD8+ and increased CD4/CD8 ratio; N/C in organ weight and CD4+ cells Thymus: Increased CD4/CD8 ratio ; Decreased organ weight and CD8SP cells; N/C in CD4SP cells	**High or Medium Confidence: counts** **NOTE: experiments in directly treated cells considered *Not informative* for these endpoints (not extracted)**
(Park et al., 2020)	Female BALB/c mice (n=10)	Fresh formaldehyde solution (methanol-free) 0, 1.38, 5.36 mg/m^3 for 2 wks (4 hr/d, 5 d/wk)	Splenic cytokines, T cell populations and Th1/Th2 balance, differentiation markers	Spleen: N/C in CD4+ T helper cells, D/D increased T reg cells (CD4+CD25+Foxp3+) subset of CD4+ cells; Increased calcinurin and NFAT1 (regulatory and inhibitory functions), N/C in NFAT2 Spleen (ex vivo production): D/D decreased IL-4, IL-5, IL-13, IFN-g, IL-17A, and IL-22 with similar changes in mRNA for same; [also, N/C in relative spleen wt. and increased rel. lung wt. at 5.36 mg/m^3]	**High or Medium Confidence [small sample size]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Dean et al., 1984)	Female B6C3F1 mice (n=6–10/ group/ endpoint, except n=5 for splenocyte assays)	PFA 18.5 mg/m^3 for 21 d (6 hr/d, 5 d/wk)	Lymphoid organ weights/ cellularity Host immunity response	N/C in thymus or spleen weight; N/C in BM cells/ femur or spleen cell counts; N/C in CFU in spleen or BM; N/C in splenic lymphocyte proliferation or splenic B cell IgM production N/C in cell-mediated immunity (response of spleen lymphocytes to mitogens, splenocyte cell surface markers, NK cell cytotoxicity) or humoral immunity (number of IgM Ab-producing B cells for 3 separate antigens) Decreased host susceptibility to bacteria challenge, but not tumor challenge; N/C in hypersensitivity or NK cytotoxicity	**Low Confidence [excessively high levels small sample size; some experiments ex vivo]** NOTE: 60–70% RB inferred
(Liu et al., 2017)	Male ICR mice (n= 10/group)	Unspecified test article 0, 1, 10 mg/m^3 for 20 wk (2 hr/d)	Bone marrow (BM) polychromatic erythrocytes (PCE)/normochromatic erythrocyte (NCE) ratio	Dose-dependent decrease in BM PCE/NCE ratio (markers of immature/mature RBCs), significant at ≥1 mg/m^3	**Low Confidence [presumed formalin]**
(Ye et al., 2013)	Male Balb/c mice (n≥9/ group/ endpoint)	Formalin 0, 0.5, 1, or 3 mg/m^3 for 7 d (8 hr/d)	ROS (dichlorohydro-flourescein and MDA) and GSH in BM and Spleen	Dose-dependent decrease in GSH levels in BM and spleen at ≥1 Dose-dependent increase in DCFH and MDA in BM and spleen at ≥1 Co-administered GSH attenuated effects on GSH, DCFH and MDA in all tissues	**Low Confidence [formalin]**
(Zhang et al., 2013)	Male Balb/c mice (n=9)	Formalin 0, 0.5, or 3 mg/m^3 for 2 wk (8 hr/d, 5 d/wk)	BM ROS and cytokines/ factors	BM increased megakaryocytes at ≥0.5 FA BM ROS (DCFH-DA) D/D increased at ≥0.5 FA; GSH decreased, and caspase-3 increased, at 3 FA; BM NFkB, TNFα, and IL-1β increased at 3 FA	**Low Confidence [formalin]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Zhao et al., 2020)	Male Balb/c mice (n=3, pooled into single sample for nose and lung samples); 2 experiments by different researchers	Formalin 0, 3 mg/m^3 for 2 wks (8 hr/d, 5 d/wk)	Burst-forming unit-erythroid (BFU-E), and colony-forming unit-granulocyte macrophage (CFU-GM) colonies in nose, lung, spleen, and bone marrow	Spleen results: Decreased formation of CFU-GM in both experiment I and II Decreased formation of BFU-E in experiment II; N/C in experiment I	**Low Confidence [formalin; small sample size]** **Not Informative: ex vivo results**
(Gu et al., 2008)	Female Balb/c and C3H/He mice (n=10 for in vivo; n=3 ex vivo experiments)	Formalin (assumed; test article NR) 0.098 mg/m^3 for 5 wk (in vivo); 0.12, or 0.98 mg/m^3 for 5 wk (ex vivo); both 24 hr/d, 5 d/wk	Splenic cell phenotypes Ex vivo cytotoxicity	N/C in T cell or B cell subtypes at 0.08 Increased NK1 cells (NK1.1 expression) at 0.098 mg/m^3 Increased ex vivo NK1 cell cytotoxicity at ≥0.12 mg/m^3	**Low Confidence [formalin]** **Not Informative [small sample size; ex vivo; unclear reporting: ex vivo cytotoxicity**
(Dallas et al., 1987)	Male SD rats (n=2/ time point; unclear reporting)	PFA 0, 0.62, 3.69, or 18.5 mg/m^3 for 1 wk to 24 wk (6 h/d, 5 d/wk)	Flow cytometry DNA/RNA analysis of BM cell proliferation/ health	N/C in RNA or DNA measures (e.g., % S phase) in BM cells	**Low Confidence [small sample size; unclear reporting]** NOTE: indirect utility for evaluating respiratory effects or inflammation
(Kim et al., 2013b)	Female NC/Nga (atopic-prone) mice (n=5–6/group)	Formalin (assumed; test article NR) 0, 0.25, 1.23 mg/m^3 for 4 wk (6 hr/d, 5 d/wk) Sensitization: topical house dust mite (HDM; ear) stimulation (25 mg Df ointment) 1×/wk for 4 wk	Cytokine mRNA for spleen	Spleen mRNA: FA D/D increase IL-13 only With HDM, FA exacerbated IL-4 (0.2), IL-5 (1.23 mg/m^3), IL-13 and IL-17A (≥0.25 mg/m^3), but caused D/D decreased IFNγ (≥0.25 mg/m^3)	**Low Confidence [small sample size; unclear reporting]** NOTE: indirect utility for evaluating respiratory effects or inflammation
(Kim et al., 2013a)	Female C57BL/6 mice (n=5 "experiments"; number of mice/ group unclear)	Formalin (assumed; test article NR) 0, 6.15, or 12.3 mg/m^3 2–3 wk (6 hr/d, 5 d/wk)	Spleen and bone marrow cell counts Ex vivo cellular functional assays	N/C in absolute cell number or T cell or B cells subtypes in spleen or BM; No significant changes in %CD8 or % B cells in spleen Decreased NK1 cells in spleen, including reduced function, which was inhibited at 12.3 mg/m^3: duration-dependent	**Low Confidence [formalin; unclear, low sample size; high levels]** **Not Informative: ex vivo function**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Yu et al., 2014b)	Male ICR mice (n=6)	Formalin 20, 40, 80 mg/m^3 for 15 d (2 hr/d)	BM histology, cell counts and ROS	Decreased BM cells observed by pathology and GSH-Px activity at ≥40 FA Increased MPO activity and protein and decreased Prx2 protein at ≥20 FA Decreased BM cells (karyocytes) and CFUs and MMP levels at 80 mg/m^3 D/D increased BM oxidative stress (MDA increased and SOD decreased) ≥20 FA Increased BM apoptosis markers ≥40 FA	**Low Confidence [formalin; excessively high levels; short periodicity]**
(Yu et al., 2015a)	Male mice (strain NR; n=6/ group)	Formalin 0, 20, 40, 80 mg/m^3 for 15 d (2 hr/d)	BM H_2O_2 production, caspase and antioxidant enzyme levels/ activity, and apoptosis	Increased ex vivo caspase-3 activity, peroxiredoxin levels and H_2O_2 production at ≥20 mg/m^3 Increased apoptosis at ≥40 mg/m^3	**Low Confidence [formalin- excessively high levels; short periodicity]**
(De Jong et al., 2009)	Male Balb/c mice (n=6)	Formalin 3.6 mg/m^3 nose-only (up to 360 min/d for 3 d)	Ex vivo cytokine production from isolated lymph nodes	No cell proliferation in LNs N/C in IL-4, IL-10, or IFNγ production from isolated cells by FA alone, but FA with sensitization results in increased IL-4 and IL-10 (and slight increase in IL-12), but N/C in IFNg	**Low Confidence [formalin; short duration and periodicity; ex vivo]**
(Zhang et al., 2014a)	Balb/c mice (n=3/sex/group)	Formalin 0, 4, 8 mg/m^3 for 7 d (6 hr/d)	Spleen and thymus weights Ex vivo spleen cell lymphocyte proliferation and ROS Urine metabolomics	Decreased relative spleen and thymus weights (only statistically significant for thymus at 8 mg/m^3) Decreased ex vivo lymphocyte proliferation and SOD activity at ≥4 mg/m^3 and increased ex vivo ROS at 8 mg/m^3	**Low Confidence [formalin; ex vivo; no chamber control exposure; lowest tested exposure of 4 mg/m^3]** **Note: some ex vivo assays after in vivo exposure; n=6 (pooled sexes assumed- not explicit in reporting)**
(Fujii et al., 2005)	Female Balb/c mice (n=6–10)	Formalin (assumed; test article NR) 0, 0.25 mg/m^3; exposed during elicitation (reporting unclear) or sensitization	Ex vivo lymph node cells all w/ epicutaneous trinitrochlorobenzene TNCB	During elicitation: FA increased CD4+ T cells (IL-4+: Th2, not IFNγ+: Th1), not CD8+, in draining lymph node (LN)	**Not Informative [formalin; ex vivo; reporting for some experiments unclear; No FA-only controls; short duration]**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
		(4 wk) or w/ chronic hypersensitivity		During sensitization (and in CH model): FA increased LN CD8+ T cells (N/C CD4+; CD4+CD25+/CD4+ decrease)	
(da Silva et al., 2015)	Male Wistar rats (n=6/ group)	Formalin 1% for 3 d (90 min/d); rats exposed in static chambers 5 rats/ time	Bone marrow cell counts	FA caused N/C in total bone marrow cells Note: while reduced effects were reported as reduced with laser therapy, laser therapy-only controls were not used	**Not Informative [formalin; unquantified high levels; static exposure chamber and group exposure; short duration and periodicity]**
(Ibrahim et al., 2016)	Pregnant Wistar rats (n=5 dams; 10 pups/group for experiments; design did not appear to account for potential litter effects)	Formalin 0.92 mg/m^3 from GDs 1–21: 1 hr/d, 5 d/wk	Total cells in femur lavage	Decreases in total cells by LPS were further reduced in offspring exposed to formaldehyde	**Not Informative [formalin; short periodicity; offspring comparisons do not include FA without LPS; small sample size; did not appear to account for litter effects]** **Note: effects rescued by vitamin C; effects on dam uterine tissue not included in these tables**
		Randomly assigned pups all received 5mg/kg lipopolysacharride (LPS) injections at PND 30			
(Lino dos Santos Franco et al., 2009)	Male Wistar rats (n=5)	Formalin 0, 1% for 3 d (90 min/ d)	BM cell counts	N/C in total BM cells; FA inhibited OVA-induced increases)	**Not Informative [formalin; unquantified high levels; small sample size; short duration and periodicity]**
		Sensitization: immediately post-FA, i.p. 10 µg OVA; boost 1 wk later with s.c. injection Challenge: 1 wk later with aerosolized OVA			
(Lino-Dos-Santos-Franco et al., 2011a)	Female Wistar rats (n=5)	Formalin 1% or naïve for 3 d (90 min/d), with or without ovariectomy	Bone marrow cell counts	Decreased total bone marrow cells	**Not Informative [formalin; impact of sham surgery; unquantified high levels; FA alone untested; naïve not chamber exposed; small sample size; short duration & periodicity]**
(Lino dos Santos Franco et al., 2006)	Male Wistar (n=5–6)	Formalin 1% or methanol vehicle for 4 d (30, 60, or 90 min/d)	Splenic and bone marrow cell counts	Increased total splenic cells, but total bone marrow cells unchanged	**Not Informative [formalin (MeOH controls); unquantified high levels; small sample size; short duration and periodicity;**

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
					comparisons to naïve rats rather than MeOH controls]
(Golalipour et al., 2008)	Wistar albino rats (n=7; sex N/R)	Mixture (dissection room vapor of undocumented composition) ≈1.85 mg/m^3 for 18 wk: 2 hr/d for 2 d/wk, 4 d/wk, or 4 hr/d for 4 d/wk	Spleen morphometry	Frequency-dependent increases in white pulp diameter and marginal zone diameter	**Not Informative [mixture exposure; short periodicity; poor reporting; controls do not account for co-exposures; quantitative comparisons for results NR]**

Table A-72. Effects on other tissues (data extracted for possible future consideration, but not included in the current analyses)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Fujimaki et al., 1992)	In vitro male SD Rat peritoneal mast cells (n=3+ experiments)	PFA 0, 1.23, 6.15, 12.3, 61.5 mg/m^3 for 30 min; stimuli: substance P, A23187 (increases cellular Ca2+ and NO production), and ant-rat IgE (in sensitized cells)	Peritoneal mast cell Histamine release	Enhanced histamine release stimulated by A23187 and anti-IgE at ≥ 6.15 mg/m^3; enhanced release by substance P at 61.5 mg/m^3 (note: release was inhibited by PLA2 inhibition, but not by antioxidant or dexamethasone)	Excluded (not tissues of interest) [In vitro; questionable relevance of peritoneal cells and exposure levels]
(Fujii et al., 2005)	Female Balb/c mice (n=6–10)	Formalin (assumed; test article NR) 0, 0.25 mg/m^3; exposed during elicitation (reporting unclear) or sensitization (4 wk) or w/ chronic hypersensitivity (CH)—all w/ epicutaneous trinitrochlorobenzene	Ear swelling, skin histopathology	During elicitation: FA suppressed contact hypersensitivity (i.e., decreased ear swelling and edema) During sensitization (and in CH model): FA increased swelling, edema, and mast cell infiltration	Excluded (not tissues of interest) [Formalin; reporting for some experiments unclear; No FA-only controls; endpoint relevance unclear]
(Dean et al., 1984)	Female B6C3F1 mice (n=5–10/ group/endpoint)	PFA 18.5 mg/m^3 for 21 d (6 hr/d, 5 d/wk)	Peritoneal macrophage function	N/C in peritoneal macrophage function, except: FA-increased H_2O_2 production by macrophages isolated after injection with MVE-2 and stimulation with PMA	Excluded (not tissues of interest) [Excessively high exposure levels; small sample size]

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Adams et al., 1987)	Female B6C3F1 mice (n=10)	PFA 18.5 mg/m^3 for 3 wk (6 hr/d, 5 d /wk)	Peritoneal macrophage counts and function (some in ex vivo cultures)	N/C in macrophage number or phagocytosis of antibody-covered erythrocytes; FA decreased leucine aminopeptidase expression FA increased release of ROS in response to external challenge (MVE-2 priming and PMA stimulus); N/C w/o challenge	Excluded (not tissues of interest) [Excessively high levels]
(Kim et al., 2013b)	Female NC/Nga (atopic-prone) mice	Formalin (assumed; test article NR) 0, 0.25, 1.23 mg/m^3 for 4 wk (6 hr/d, 5 d/wk) Sensitization: topical house dust mite (HDM; ear) stimulation (25 mg Df ointment) 1×/wk for 4 wk	Atopic dermatitis/ Ear skin Inflammation Cytokine mRNA for ear skin	FA increased AD-like clinical skin inflammation by HDM, but not FA alone Mast cell infiltration in dermis by FA alone, exacerbates HDM eosinophil & mast cell Skin mRNA: 0.25 mg/m^3 increased IL-13,IL-17A, COX-2; with HDM, FA exacerbated these and IFNγ, IL-4, and TSLP; N/C IL-5	Excluded (not tissues of interest) [Formalin; small sample size] Note: unclear utility for evaluating respiratory effects or inflammation; multiple supplementary files; eosinophil data not reported
(Maiellaro et al., 2014)	Pregnant Wistar rats (n=5)	Formalin 0.92 mg/m^3 from GD1–GD21: 1 hr/ d, 5 d/wk Sensitization: s.c. 10ug OVA with sc boost after 7 d Challenge: 7 d later, 1% OVA aerosol 15 min/d, 3 d	Uterine factors	Decreased uterine IL-10, SOD2, and cNOS, and increased COX-1, at birth (N/C in IL-6, IL-4, IFNγ, COX-2, iNOS, SOD1, or catalase) Decreased birth weight in offspring	Excluded (not tissues of interest) [Formalin, short duration, offspring comparisons do not include FA alone]
(Aydin et al., 2014)	Male SD rats (n=6/group)	Test article unclear, but appears to be formalin in this experiment at 0, 6.48 (low), 12.3 (moderate), or 18.7 mg/m^3 for 4 wk (8 hr/d, 5 d/wk)	Liver tissue total antioxidant and total oxidant levels (TAS and TOS; kit uses vitamin E and H_2O_2 as reference, respectively Liver tissue apoptotic index and oxidative stress index (OSI: TOS/TAS)	Increased TOS and decreased TAS, at ≥ 12.3 mg/m^3 formaldehyde Decreased irisin and increased OSI at ≥6.48 mg/m^3 Note: Carnosine supplementation reduced changes.	Excluded (not tissues of interest) [Formalin; high levels]

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Bakar et al., 2015)	Male Wistar albino rats (n=7)	i.p. Formalin every other day at 1 mg/kg/d for 14 d	Kidney biochemistry, immunoreactivity for Bcl-2 and Bax, ROS and antioxidant markers, and electron microscopy	Increased Bcl-2 and Bax immunostaining, and increased ROS markers and altered antioxidant enzyme activities; kidney damage and inflammation noted	Excluded (not tissues of interest) [Formalin; high levels of unknown comparability to inhaled levels; i.p. injection]
(Matsuoka et al., 2010)	Male ICR mice (n≥ 7)	Formalin at 0.12 mg/m^3 for up to 24 hr; also, a single experiment at 3.69 mg/m^3 for 24 hr with LPS	Urine, liver, brain ROS (8OHdG) and NO metabolites (nitrates/ nitrites)	Decreased ROS in urine and liver; N/C in brain; decreased NO in urine, liver and brain at 0.12 mg/m^3 at 24 hr Increased urinary SOD activity:3.69 mg/m^3	Excluded (not tissues of interest) [Formalin; short duration]
(Kum et al., 2007b)	Female SD rats (n=6/group) at GD1 [I], PND1 [II], PND28 [III] or adults [IV]	Formalin (assumed: test article NR): 0 or 7.38 mg/m^3 for 6 wk (8 hr/d, 7 d/wk)	Liver oxidative stress (i.e., SOT, CAT, GSH, MDA)	CAT activity and MDA levels increased [I] GSH decreased in [II] SOD activity decreased [III] N/C in adult [IV] oxidative stress markers Note: body and liver weight decreased in I and II; liver weight increased in III	Excluded (not tissues of interest) [Formalin, high levels; limited assays]
(Kum et al., 2007b)	Female SD rats (n=6/ group)	Formalin (assumed: test article NR): 0 or 7.38 mg/m^3 for 6 wk (8 hr/d, 7 d/wk);	Renal oxidative stress	N/C in renal SOD, CAT, GSH-Px, GSH, or MDA by FA alone	Excluded (not tissues of interest) [Formalin, high levels; limited assays]
(Ciftci et al., 2015)	Male Wistar albino rats (n=10)	Formalin i.p. injection at 9 mg/kg/d every other day for 2 weeks	Brain and urine oxidative DNA damage Beta amyloid in brain	Increased A-beta$_{1\text{-}42}$ in brain Increased brain DNA 8-Ohdg damage; slightly increased (nonsignificant-assumed) DNA damage in urine	Excluded (not tissues of interest) [high levels of unkown relevance; i.p. injection; formalin]
(Ye et al., 2013)	Male Balb/c mice (n≥9/ group/ endpoint)	Formalin 0, 0.5, 1, or 3 mg/m^3 for 7 d (8 hr/d)	ROS (dichlorohydro-flourescein and MDA) and GSH in Liver and Testes	D/D decrease in GSH levels in liver at ≥0.5 mg/m^3; decreased in testes at 3 mg/m^3 D/D increase in DCFH and MDA in liver at ≥0.5 mg/m^3; in testes at ≥1 mg/m^3; co-administered GSH attenuated effects on GSH, DCFH and MDA in all tissues	Excluded (not tissues of interest) [Formalin]

Study	System	Exposure	Endpoint(s)	Results	Utility and notes*
(Jiang et al., 2015)	In vitro PC12 (immortalized neuronal) cells (n=3 technical replicates)	Formalin (assumed; test article NR)—in vitro levels of unknown relevance	Viability, neurotrophic factor, and ROS markers	Decreased BDNF and viability Increased MDA and other ROS markers	Excluded (not tissues of interest) [Formalin, high levels of unknown relevance; in vitro; small sample size]
(Kim et al., 2013a)	Female C57BL/6 mice (n=5 "experiments"; number of mice/ group unclear)	Formalin (assumed; test article NR) 0, 6.15, or 12.3 mg/m^3 2–3 wk (6 hr/d, 5 d/wk)	liver cell counts Ex vivo cellular functional assays	N/C in absolute cell number or T cell or B cells subtypes in liver	Excluded (not tissues of interest) [Formalin; unclear sample size]
(Güleç et al., 2006)	Wistar albino rats (n=10; sex NR)	PFA 0, 12.3 or 24.6 mg/m^3 (8 hr/d, 5 d/wk) for 4 or 13 wk	Heart oxidative stress (i.e., SOD, CAT, TBARS, NO)	Increased SOD at ≥ 12.3 mg/m^3 (4 or 13 wk); Decreased CAT at ≥ 12.3 mg/m^3 at 4 wks, but not 13 wk; N/C in TBARS or NO	Excluded (not tissues of interest) [excessively high levels; limited assays]
(Xin et al., 2015)	HepG2 (liver) cells; n=3 technical replicates	Formalin; in vitro (unknown relevance)	Heat shock protein reporter assays	Increased promotion of HSPA1, correlated with oxidative stress and cellular damage	Excluded (not tissues of interest) [in vitro; high levels; formalin; small sample size]

1

Synthesis of the identified mechanistic evidence by tissue compartment

The most likely initial effects of formaldehyde exposure include evidence of direct interactions of formaldehyde with biological macromolecules (e.g., DNA; receptors; redox proteins) in the upper respiratory tract (URT). These direct interactions would typically not be expected to occur in other tissue compartments given the lack of substantial distribution of inhaled formaldehyde to distal sites (see Appendix A.2). While stress hormone increases likely involve prior modification of the hypothalamic-pituitary-adrenal (HPA) axis, *slight* evidence of this change is indicated as a plausible initial effect of exposure due to a general lack of knowledge of the specific type of stressor(s) (e.g., direct responses due to subtle changes in fear or anxiety; indirect effects via sustained inflammation) and the nature of the interactions with the HPA axis that might result from formaldehyde inhalation. The *slight* evidence of indirect evidence for sensory nerve stimulation in the LRT is not indicated as a plausible initial effect of exposure because inhaled formaldehyde is unlikely to reach the LRT in appreciable amounts and it is expected that LRT sensory nerve activation would be reliant on a secondary response to TRP channel-activating stimuli such as increased LRT oxidative stress or inflammatory mediators; although, certain exposure scenarios (e.g., after exposure to high levels of formaldehyde or mouth breathing during exercise, perhaps only in susceptible individuals) might, in rare scenarios, result in distribution of minimal amounts of formaldehyde to upper regions of the LRT (see Appendix A.2) that may be sufficient to induce such receptor-mediated events. Although it is difficult to disentangle the multiple mechanistic events manifested soon after formaldehyde inhalation, it appears that formaldehyde can initiate overlapping events in the URT, including effects at the level of the respiratory epithelial cells and overlying mucociliary layer, as well as at trigeminal nerve endings. While uncertainties remain[18], the effects in the lower respiratory tract (LRT), blood, and other organs are likely secondary to the changes observed in the URT. Figures A-31 and A-32 illustrate the potential relationships between the mechanistic events reported from formaldehyde exposure, based on the more reliable evidence (see Figure A-31) or including evidence that should be interpreted with greater caution (see Figure A-32). These figures are based on evidence summarized in Tables A-66 to A-72, and they are discussed according to tissue compartment in the sections below.

Figures A-31 and A-32 (on the following pages) present network summaries of mechanistic data related to potential noncancer respiratory health effects of formaldehyde. These figures present an integrated picture of the mechanistic events identified from studies of formaldehyde exposure. The figures are organized by tissue type or region (i.e., upper respiratory tract, "URT";

[18] Controlled human exposure studies observed pulmonary function deficits when a longer exercise component (15 minutes) was included. These deficits were not observed by other studies with shorter periods or no exercise (Green et al., 1989; Green et al., 1987), and another study observed airway hyperresponsiveness with an exposure protocol using nose clips requiring mouth-only breathing (Casset et al., 2006).

lower respiratory tract, "LRT"; "blood"; and other tissues related to immunological responses, "other"), the data for which are summarized in the following subsections. Figure A-31 presents events interpreted with greater confidence (i.e., *robust* or *moderate* evidence), while Figure A-32 includes events based on *slight* evidence. In both figures, the mechanistic events and the relationships between events are characterized as defined in Table A-64. Lines with arrows on both ends indicate events for which the association appears to be bidirectional. The figures also identify events that are "plausibly an initial effect of exposure," and each event is related to one or more "key features of a potential hazard" (see explanations above). Note: Some events and relationships are not shown for clarity, but nearly all mechanistic events from Tables A-66 to A-72 for which at least *slight* evidentiary support was concluded are presented. Note that "decreased pulmonary function" encompasses a range of possible contributing effects including, but not limited to, increased bronchoconstriction, flow limitation, and decreased bronchodilation.

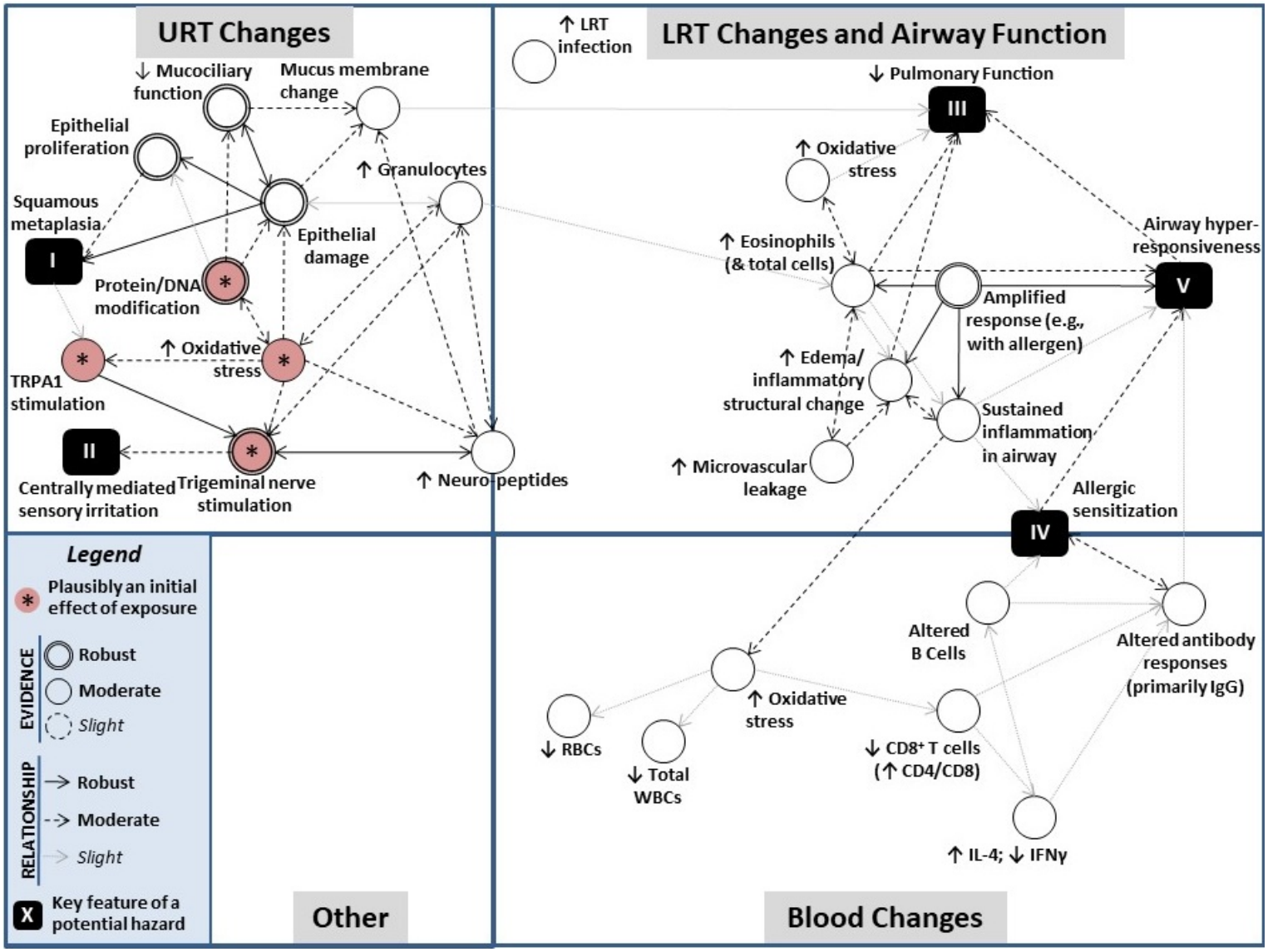


Figure A-31. Mechanistic events for respiratory effects of formaldehyde based on *robust* or *moderate* evidence.

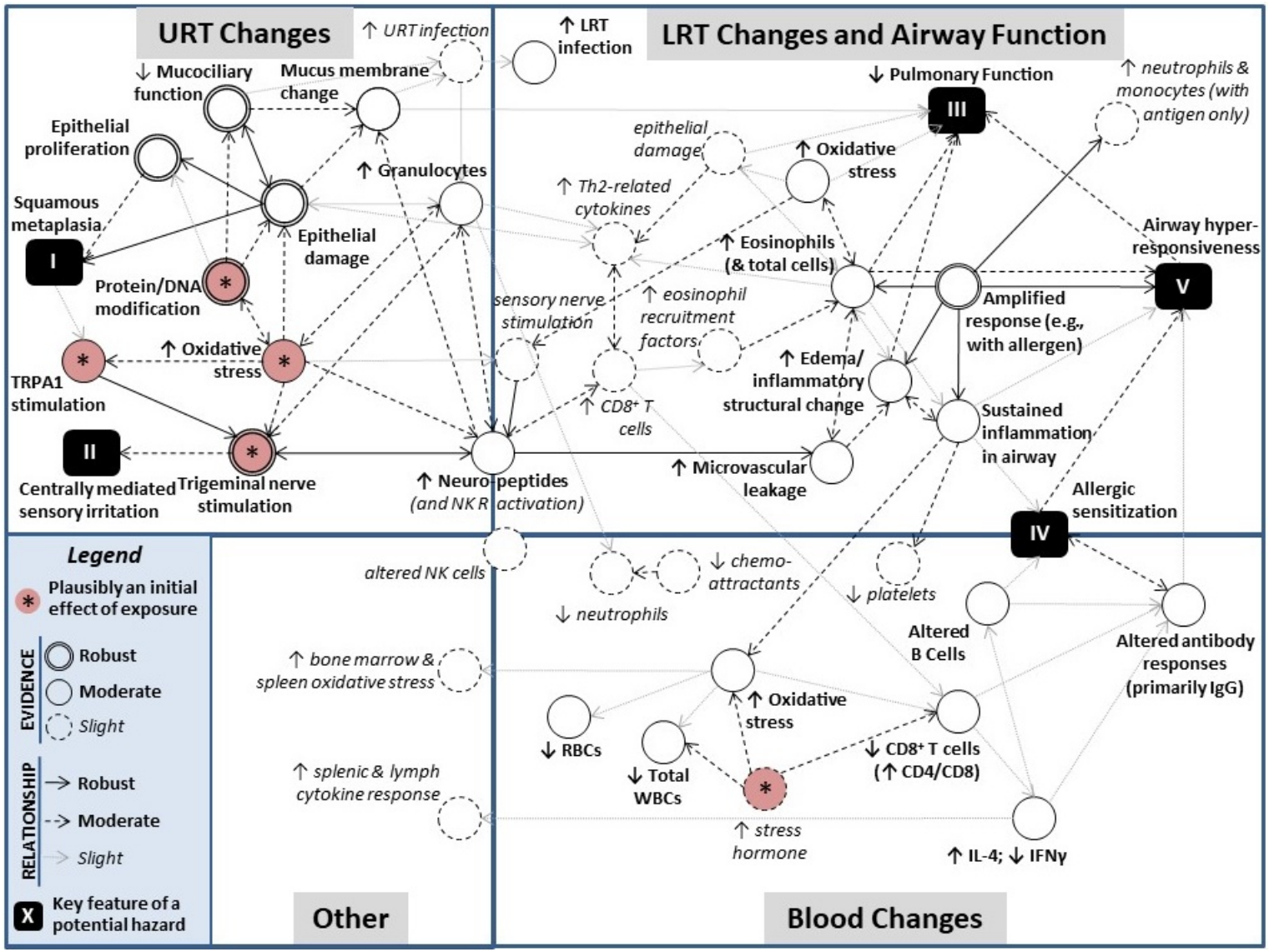


Figure A-32. Mechanistic events for respiratory effects of formaldehyde based on *robust*, *moderate*, or *slight* evidence.

Changes in the URT

Data on formaldehyde-induced mechanistic changes in the URT are largely based on studies in experimental animals or acutely exposed human volunteers, as most of these endpoints are difficult to examine in long-term observational epidemiology studies. The specific studies and summary findings supporting the synthesis below are described in Table A-73. While the structure and function of the URT across species is considered similar, interpretation of compensatory or adaptive changes within the human URT following long-term exposure based on findings in experimental animals is difficult to infer.

The majority of the events which are potential initial or direct effects of formaldehyde (see asterisks in Figure A-31) occur at the level of the respiratory epithelium, including evidence supporting the involvement of formaldehyde in reactions with cellular macromolecules such as proteins (e.g., detoxifying enzymes) and DNA, effects on the local redox system, and interactions with sensory nerve endings within the respiratory epithelium. While these events are interrelated, they could be caused by formaldehyde independently and simultaneously. Although some studies have reported changes in these initial mechanistic events at formaldehyde concentrations as low as 0.035 mg/m^3 following acute or short-term exposure, notable uncertainties remain. For example, tissue alterations that might increase vulnerability to these changes with continued exposure is expected (e.g., decreases in mucociliary clearance). Conversely, gradual tissue changes following exposure might also lead to resilience (e.g., increases in epithelial cell barrier function). More detailed mechanistic studies characterizing the initial molecular interactions of formaldehyde in the URT following long-term exposure would help to clarify potential progressive changes in the ability of formaldehyde inhalation to elicit these intial changes.

Effects on the mucociliary system are likely secondary to the production of reactive byproducts or covalent modification to mucosal structural components following physical interactions of formaldehyde with proteins in the mucus. The effects of formaldehyde on mucus flow patterns appear to include both a concentration and exposure-duration dependency (as well as variability due to humidity), although a mechanism reliant on direct modification of macromolecules alone would be expected to be driven largely by concentration. The impact of this is difficult to define and integrate into the overall mechanistic picture. Persistent changes to the normally protective mucociliary apparatus or tissue redox capacity are likely to eventually lead to epithelial damage (which has been shown to correlate with inhibited mucociliary function following formaldehyde exposure). To repopulate damaged tissue and cells, and to protect against further insult, damage often leads to cell proliferation or hyperplasia (i.e., an increase in the amount of tissue due to proliferation of normal cells), and/or the damage can eventually lead to epithelial lesions such as squamous metaplasia, where cells transition to a different phenotype. This proliferation, hyperplasia, and/or metaplasia can be adaptive (e.g., response to tissue stress) or maladaptive, and could lead to subsequent effects on pulmonary function through thickening or keratinization of the respiratory epithelium, or thickening of mucus, all of which can restrict

airflow. Formaldehyde exposure-induced damage to the URT epithelial cells could also result in an altered release of cytokines or other soluble mediators, which, were they to reach the LRT, could contribute to decreased pulmonary function through airway hyperreactivity and/or hypersensitivity to challenges such as allergen exposure (Hulsmann, 1996, 3266586). In general, the plausible initial mechanistic events and changes in mucus flow patterns observed after formaldehyde exposure occur at lower formaldehyde levels than those eliciting URT epithelial lesions (i.e., at ≤0.3 mg/m^3 in exposed humans and >0.6 mg/m^3 in animals).

Inhaled formaldehyde also appears to directly stimulate trigeminal nerve endings in the nasal mucosa. Activation of these chemosensory afferents, likely C fibers, is known to initiate afferent signals that result in the burning sensation characteristic of sensory irritation. This chemosensory activation is enhanced in the anterior third of the nasal cavity and is typically less sensitive than olfaction (Hummel and Livermore, 2002). These characteristics are consistent with the known distribution of inhaled formaldehyde (see Appendix A.2) and with observations that formaldehyde exposure typically causes chemosensory-related irritation at higher concentrations than those necessary for olfactory detection in naïve individuals (e.g., as demonstrated by e.g., as demonstrated by Berglund et al., 2012). The rapid detection of these sensations in exposed individuals suggests a receptor-mediated event that is dependent on formaldehyde penetration to the nerve endings, which may not have an exposure duration threshold. Based on mechanistic studies in vitro and ex vivo, activation of the trigeminal nerve by formaldehyde is likely mediated, at least in large part, through Transient Receptor Potential A1 (TRPA1) cation channels. To a lesser extent, this activation may also involve TRPV1 channels, which can be coexpressed and coactivated alongside TRPA1 in certain situations (Salas et al., 2009). Overall, very little is known about changes in chemosensitivity to inhaled formaldehyde with repeated exposure over time, as mechanistic studies of long-term exposure were not identified. With acute, controlled exposure in human volunteers, the initial irritation response to formaldehyde, which is highly variable across individuals, has been shown to plateau (e.g., e.g., Green et al., 1987) or even decline somewhat (e.g., e.g., Bender et al., 1983) when exposure is continued for several minutes to hours; however, this pattern may depend upon concentration (Andersen and Molhave, 1983), and changes to this response pattern in humans over time, particularly with exposure longer than 1 day, remain unclear. Studies of reflex bradypnea in rodents (see Appendix A.3), which is dependent on the activation of the trigeminal nerve, show that repeated exposure for up to a month elicits a similar level of activation of this pathway. However, uncertainties with these data include a nonconstant exposure (i.e., short-term rodent studies employed work hour-like exposure periodicity) and testing only at reflex bradypnea-inducing levels (e.g., >1 mg/m^3). It is unclear how this informs long-term responses to constant oronasal exposure in humans (who do not exhibit this reflex) at lower formaldehyde levels. Enhanced irritation with prolonged exposure could occur directly as a result of sensitization of the receptors (e.g., TRPA1) to formaldehyde or indirectly by increased access of formaldehyde to trigeminal nerve endings following damage to juxtaposed epithelial cells.

Electrophilic oxidative stress products such as hydrogen peroxide and 4-hydroxynonenal are also known to be capable of stimulating sensory nerve receptors such as TRPA1 (Taylor-Clark et al., 2008; Alexandersson, 1988), and *moderate* evidence exists to support the presence of oxidative stress in both the upper and lower airways. In addition, airway inflammation has been shown to reduce the threshold for activation of afferent fibers, through an unknown mechanism (Carr and Undem, 2001). Conversely, however, as this action is mediated predominantly by access of formaldehyde to chemoreceptors, changes such as the conversion of normal epithelium to squamous epithelium or damage and destruction of nerve afferents would be expected to reduce or desensitize subsequent irritant responses. Taken together, this suggests a complex sequence of interactions that might impact trigeminal nerve chemosensation over time.

Together with the centrally mediated physiological response, stimulation of airway sensory nerves, including the trigeminal nerve, can also cause a more immediate localized release of neuropeptides like substance P and calcitonin gene-related protein (CGRP). These released neuropeptides, particularly substance P, can affect local immune responses by increasing vascular permeability and leukocyte recruitment, among other things (Sarin et al., 2006), as has been demonstrated with substance P-dependent eosinophil accumulation in the human nasal mucosa after allergen exposure (Fajac et al., 1995). Observations of neuropeptide changes, including increased substance P, have been reported at slightly higher formaldehyde levels than those shown to activate the trigeminal nerve, generally >1 mg/m^3. While URT neuropeptide levels have not been examined in great detail following formaldehyde exposure, given that the URT represents the primary region of formaldehyde flux, formaldehyde exposure-induced increases in neuropeptides in model systems and related tissue regions, including the LRT, are inferred to provide support for the few URT-specific studies that observed elevated neuropeptide levels. The formaldehyde-specific data further indicate that the neuropeptides are released from neuronal rather than nonneuronal sources, at least following short-term exposure, and this release appears to be at least partially dependent on TRPA1 activation. The formaldehyde-specific URT studies have not examined many of the potential consequences of these changes, particularly after long-term exposure. Elevated URT neuropeptides might result in local inflammatory changes ranging from increased histamine and mucus secretion to edema and nasal obstruction during normal or exaggerated attempts to minimize nasal irritation (Barnes et al., 1991a, b).

The immune response in the URT following formaldehyde exposure has not been thoroughly studied, particularly in exposed humans; however, the available evidence does provide *moderate* support for granulocyte (e.g., eosinophils; neutrophils) involvement. The available data generally indicate that eosinophils are increased in the URT with acute or short-term exposure at ≈0.5 mg/m^3, although one study suggests the possible increases at much lower levels in exposed humans with longer exposure (Norback et al., 2000). Although the role for eosinophils in the upper airways of exposed individuals remains unclear, airway eosinophils are known to be tightly regulated and uncommon in normal airways. In addition to their traditional role as immune

"effectors" (i.e., releasing toxic molecules to destroy invading pathogens), activation of eosinophils can also cause them to release a number of chemical mediators which damage epithelial cells, stimulate mucus secretion, induce airway hyperresponsiveness, and perpetuate further recruitment of inflammatory mediators into the airway (Cohn et al., 2004). Eosinophils, which are relatively rare (≈1%) blood leukocytes, are a hallmark of allergic asthma (Howarth et al., 2000); however, no formaldehyde-specific studies meeting the inclusion criteria evaluated the URT for changes in other commonly observed inflammatory markers of allergic individuals such as activated mast cells or histamine. In addition, the data are unable to inform whether this inflammatory change persists in the human URT with long-term exposure. It should be recognized that acute inflammation is a protective response of the tissue to stress or damage; inflammation is more concerning when it becomes dysregulated, recurrent, and/or persistent.

At much higher concentrations (>5 mg/m^3), neutrophils also appear to increase within the upper airways, presumably via migration from the blood. Neutrophils, which are the most common (≥50%) blood leukocyte, are also relatively uncommon (≤2%) in healthy airways. These phagocytic cells, along with eosinophils, are one of the first cells recruited to inflamed tissues shortly after infection. Both eosinophils and neutrophils can release toxic mediators, including lipid-active factors and reactive oxygen species (ROS), for which *moderate* evidence exists to support increased levels in the URT following formaldehyde exposure, and can damage bystander epithelial cells. However, in contrast to eosinophils, neutrophils are not thought to be associated with allergic responses or asthma, although they can be increased in individuals with pulmonary disease (O'Donnell et al., 2006). Changes in other cells in the URT, including basophils, macrophages, and lymphocytes, were not observed in the few short-term studies examining them.

Exactly how or why eosinophils and neutrophils migrate to the upper airways following formaldehyde exposure remains unclear. One possibility is that this response is related to the *slight* evidence of increased frequency and duration of URT infections in chronically exposed humans. However, while this effect might be caused by loss of barrier function (e.g., from epithelial cell damage or inhibited mucociliary function) leading to increased colonization of the epithelium by bacteria, this is not temporally plausible for the eosinophil increases observed following acute exposure. Evidence of specific changes in chemoattractants known to stimulate recruitment of these cells to the URT (e.g., eotaxin; IL-5; or, indirectly, TNFα or IL-1β, which can stimulate eotaxin in epithelial cells) was not identified, and thus, the biological explanation for the recruitment of these cells to the upper airways is unknown. Although not examined, it is also possible that formaldehyde could directly or indirectly (e.g., through tissue damage) interact with and modify epithelial components, including pattern recognition receptors, that can trigger release of ROS and lead to immunological responses (Kim et al., 2015a; Lambrecht and Hammad, 2012). Overall, although *moderate* evidence indicates that inflammatory cells including eosinophils and neutrophils are increased in the URT following formaldehyde exposure, the data are limited in their

ability to define the concentration and duration requirements for the effects of formaldehyde exposure on URT immunological processes, which might inform how these changes are initiated.

Table A-73. Summary of changes in the upper respiratory tract (URT) resulting from formaldehyde exposure

Endpoint		Study-specific findings from "high or medium" or "low" confidence experiments	Summary of evidence (exposure duration)	Conclusion
Structural Modification of the Upper Airways				
Modification of biological macromolecules [see Appendix A.2 and A.4 for additional detail]	High or Medium	*Human:* None (note: binding of formaldehyde to albumin and other soluble proteins in human mucus has been demonstrated in vitro; e.g., Bogdanffy et al. (1987); hemoglobin adducts at ≈0.2 mg/m^3, Bono et al. (2012)	Consistent with its known chemistry, formaldehyde can modify cellular biological macromolecules, including DNA, and interacts with soluble factors such as albumin and glutathione, shortly after exposure to low concentrations (e.g., <0.5 mg/m^3) across a wide range of exposure durations	**Robust**
		Animal: Multiple animal studies demonstrate that inhaled formaldehyde can bind and modify biological macromolecules, which is consistent with the known biological reactivity of formaldehyde; evidence includes increased DNA-protein crosslinks (DPXs), hydroxymethyl (hm) DNA adducts, and reactions with glutathione; (e.g., increased DPXs are observed at ≥0.37 mg/m^3, Casanova et al. (1989); hmDNA adducts and protein adducts at ≥0.86 mg/m^3, (Edrissi et al., 2013b; Lu et al., 2011; Lu et al., 2010a)		
	Low	*Human*: N/A (see summary)	Sufficient information for 'Robust' from *high or medium confidence* studies	
		Animal: N/A (see summary)		
Impaired Mucociliary Function	High or Medium	*Human*: decreased mucus flow at ≥0.3 mg/m^3 after acute exposure and pathological changes in mucociliary clearance in workers at mean exposed levels of 0.25–0.26 mg/m^3 after chronic exposure (Holmström and Wilhelmsson, 1988; Andersen and Molhave, 1983).	Decreased mucus flow and ciliary beat, and impaired clearance, in humans and rats at ≥0.25 and ≥2.5 mg/m^3, respectively (observed across exposure durations), eventually leading to cilia loss	**Robust**
		Animal: mucociliary function was generally unaffected at 0.57 mg/m^3 after short-term exposure—minor changes were notable at 2.46 mg/m^3; robust changes were observed at the next highest concentrations tested, ≥7.27 mg/m^3; a general lack of recovery with longer exposure duration		
	Low	*Human*: Increases in ciliary activity at 1.23 mg/m^3 in dissociated human nasal epithelial cells (Wang et al., 2014b), with decreased cilia beating frequency in human epithelial cells at ≥3.46 mg/m^3 (Wang et al., 2014b; Schafer et al., 1999): in vitro acute	Suggestive of decreased ciliary beat and ciliastasis at ≥5 mg/m^3 in humans and rats with acute	

Endpoint	Study-specific findings from "high or medium" or "low" confidence experiments		Summary of evidence (exposure duration)	Conclusion
		Animal: Ciliastasis and mucostasis: (Morgan et al., 1986c) acute 14.76 mg/m^3 (not ≤2.46 mg/m^3; recovery); Morgan et al. (1984): acute in vitro (frog palates) ≥5.36 mg/m^3 (authors noted early activity increase, even at 1.69 mg/m^3); structural cilia changes: (Monteiro-Riviere and Popp, 1986) short-term ≥0.5 mg/m^3, (Abreu et al., 2016) acute at 0.25, but not 1.2–3.7 mg/m^3	exposure, and cilia damage at ≥0.5 mg/m^3 with short-term exposure; usually preceded by initial effects including slight increases in activity	

Table A-73. Summary of changes in the upper respiratory tract (URT) resulting from formaldehyde exposure (continued)

Endpoint		Study-specific findings from "high or medium" or "low" confidence experiments	Summary of evidence (exposure duration)	Conclusion
Structural Change in URT Mucus Membrane or Nasal Obstruction	High or Medium	*Human*: Membrane hypertrophy, atrophy, rhinitis: (Lyapina et al., 2004) chronic (yrs) 0.87 mg/m^3	Mucus membrane damage and swelling in humans at 0.87 mg/m^3 with chronic exposure	**Moderate** particularly in persons with nasal damage
		Animal: None		
	Low	*Human*: Data suggest increased mucosal swelling, nasal obstruction, and/or rhinitis in workers (Holmström and Wilhelmsson, 1988) chronic at 0.26 mg/m^3 and (Norback et al., 2000): short-term at ≤0.016 mg/m^3, which did not increase in severity with longer exposure; increase in mucosal swelling in symptomatic nasal distress patients, but not healthy controls: Falk et al. (1994) acute (2 hr) ≥0.073 mg/m^3	Observations at ≤0.26 mg/m^3 in humans or at >3.5 mg/m^3 in rats support data from the chronic-duration study and suggest increased acute vulnerability of people with a prior nasal condition	
		Animal: Rhinitis and necrosis in rats after acute or short term (1−3 d) at ≥3.94 or 4.43 mg/m^3		
URT Epithelial Damage or Dysfunction [see Toxicological Review Section 1.2.4 for additional data and discussion]	High or Medium	*Human*: Indirect data indicating epithelial damage, including loss of ciliated cells, in occupational studies at 0.1−>2 mg/m^3 (Holmström and Wilhelmsson, 1988), 1989, 3564; Edling et al. 1987, 4059 (Ballarin et al., 1992; Edling et al., 1988), with one with more equivocal findings (Boysen et al., 1990); however, these histopathological symptom scores included hyperplasia and metaplasia, which complicate interpretation	Duration-dependent epithelial damage, typically at ≥2.5 mg/m^3 in subchronic or chronic rat studies, and with supportive indirect findings from human studies at 0.1−0.2 mg/m^3, generally correlates with inhibited mucociliary activity	**Robust**
		Animal: Increased epithelial damage and related nasal lesions: duration-dependent, typically ≥2.46 mg/m^3 in subchronic and chronic studies (e.g., (Andersen et al., 2010) lower in some longer-term studies) and generally correlating with inhibited mucociliary activity; goblet cell loss in monkeys (Monticello et al., 1989) short term (1 wk) at 7.38 mg/m^3		
	Low	*Human*: None	Studies suggest that nasal epithelial damage is increased, even in short-term studies, at ≥2.5 mg/m^3	
		Animal: Goblet cell damage and decreased junctional proteins between epithelial cells in rats (Arican et al., 2009): subchronic (12 weeks) at 18.5 mg/m^3; mRNA and/or miRNA changes associated with apoptosis (Rager et al., 2014): short term (2 d in macques or 28 d in rats) or DNA repair (Andersen et al., 2010): short term (1 wk, but not at 4−13		

Table A-73. Summary of changes in the upper respiratory tract (URT) resulting from formaldehyde exposure (continued)

Endpoint	Study-specific findings from "high or medium" or "low" confidence experiments		Summary of evidence (exposure duration)	Conclusion
		week durations) at ≥2.46 mg/m^3; Rhinitis and necrosis in rats after acute or short term (1−3 d) at ≥3.94 or 4.43 mg/m^3		
URT Cellular (Epithelial) Proliferation [see Toxicological Review Section 1.2.4 for additional data and discussion]	High or Medium	*Human*: None: indirect data from humans indicating an increase in histopathological scores that sometimes included hyperplasia were not specific enough to independently evaluate proliferation	Increased cell proliferation in rats at all tested durations. Proliferation increases were typically observed in the anterior nasal cavity at tested levels ≥≈3.5−4 mg/m^3, and were generally not observed at ≤1.23 mg/m^3. Sites of proliferation correlated with the development of hyperplasia and metaplasia, although the temporal and exposure levels specifics of this association are unclear. Indirect data from observations of hyperplasia in exposed animals and humans are consistent with these data.	**Robust ↑**
		Animal: Acute dose-dependent increases in cell proliferation in rats, measured primarily by DNA labeling during the final days of exposure, were consistently observed following acute, short-term, and subchronic exposure, and generally with a similar magnitude of responses across durations. Proliferation was typically highest in anterior regions (e.g., "level 2"), with little evidence of proliferation at ≤1.23 mg/m^3, mixed findings between 1.24 and 3.5 mg/m^3, and studies generally reporting increases with exposure at higher levels, particularly with longer exposure duration. These data are supported by consistent observations of formaldehyde exposure-induced increases in hyperplasia in pathology studies, some of which provided information showing a correlation between acute proliferation and hyperplasia and metaplasia. The only rat study that measured exposure longer than 13 wks suggests that increases in acute proliferation may begin to decrease in magnitude with chronic exposure at ≥6 mg/m^3 (Monticello et al., 1996). A few studies suggest that mice may exhibit less robust responses than rats, while monkeys may exhibit proliferation in more posterior nasal regaions at >7 mg/m^3.		
	Low	*Human*: N/A (see summary)	Sufficient information for 'Robust' from *high or medium confidence* studies	
		Animal: N/A (see summary)		
Sensory Nerve-Related Changes				
Trigeminal Nerve Stimulation	High or Medium	*Human*: None	Increased activity of trigeminal nerve afferents at <0.5 mg/m^3 following acute exposure in animals	**Robust ↑**
		Animal: Increased afferent nerve activity: Tsubone and Kawata (1991) acute ≈20% at 0.62 mg/m^3 and ≈50% at 2.21 mg/m^3; Kulle and Cooper (1975) acute (threshold detection at 25 sec) at 0.31 mg/m^3		

Table A-73. Summary of changes in the upper respiratory tract (URT) resulting from formaldehyde exposure (continued)

Endpoint	Study-specific findings from "high or medium" or "low" confidence experiments		Summary of evidence (exposure duration)	Conclusion
	Low	*Human*: None *Animal*: Indirect evidence: with acute exposure, dose-dependent increase in nerve currents and Cl—release in intact rat trachea (Luo et al., 2013), and stimulation using in vitro neuronal preparations (Kunkler et al., 2011; Mcnamara et al., 2007)	Supportive indirect evidence from ex vivo and in vitro experiments	
TRPA1 and/or TRPV1 Stimulation	High or Medium	*Human*: None *Animal*: Formaldehyde and related chemicals such as acrolein activate the trigeminal system in wild-type mice, but not TRPA1 knockout mice following acute exposure, at least at high exposure levels (Yonemitsu et al., 2013); taken together with the established role for TRPA1 in acrolein-induced sensory effects (e.g., e.g., Bautista et al., 2006); these data indirectly support a role for TRPA1 in sensory nerve-related changes following formaldehyde exposure	Indirect data identify TRPA1 as a molecular target for formaldehyde exposure-induced sensory effects	**Moderate (TRPA1); Minimal (TRPV1: not shown in figures)**
	Low	*Human*: None *Animal*: Formaldehyde activates the transient receptor potential cation channels, TRPA1 and TRPV1, in in vitro and ex vivo models relevant to acute inhalation exposure of the URT and upper LRT: (Luo et al., 2013; Mcnamara et al., 2007), and in vivo using formalin as a pain stimulus (not shown); Inhibition of TRPA1 and TRPV1 channels localized to sensory nerve endings reduce FA exposure-induced nerve currents in rat trachea (Luo et al., 2013) and immune-related responses in mice (Wu et al., 2013; Lu et al., 2005): 1 or 3 mg/m^3 for 2 or 4 wk	Indirect data identify TRPA1 and/or TRPV1, as molecular target(s) of formaldehyde exposure with acute or short-term exposure; inhibitor studies demonstrate that downstream effects of sensory nerve stimulation depend on TRPA1 or TRPV1 stimulation.	
Neuropeptide Release	High or Medium	*Human*: None *Animal*: **in plasma:** Increased substance P in mice with subchronic exposure (Fujimaki et al., 2004b): subchronic at 2.46 mg/m^3	Indirect evidence that Substance P was increased with subchronic exposure in a single mouse study at 2.46 mg/m^3	**Moderate ↑ (relevant to both URT and LRT; note: evidence for**
	Low	*Human*: **in URT:** Substance P in nasal lavage is increased in human volunteers with ocular exposure (He et al., 2005): 4 d (5 min/d) at 3 mg/m^3, but not at 1 mg/m^3	Data suggest formaldehyde activates TRP channels on sensory neurons,	

Table A-73. Summary of changes in the upper respiratory tract (URT) resulting from formaldehyde exposure (continued)

Endpoint	Study-specific findings from "high or medium" or "low" confidence experiments		Summary of evidence (exposure duration)	Conclusion
		Animal: **in URT:** Formaldehyde stimulates release of calcitonin gene related-protein (CGRP) in in vitro models relevant to inhalation exposure of the URT (Kunkler et al., 2011); Experiments using the related chemical, acrolein, suggest this is TRPA1-mediated (Kunkler et al., 2011). **in LRT:** Inhibition of substance P receptor (NK1) inhibited formaldehyde-induced currents in isolated rat trachea (Luo et al., 2013); increased substance P and CGRP in mouse BAL, both amplified with ovalbumin (OVA) sensitization, and both involved TRP activation (Wu et al., 2013): short term at 3 mg/m^3	leading to release of CGRP and substance P, with acute or short-term exposure at >1 mg/m^3	**NK Receptor involvement is Slight)**
Immune and Inflammation-Related Changes				
URT Oxidative Stress	High or Medium	*Human*: Increased nasal epithelial M1dG adducts (marker for oxidative stress and lipid peroxidation (Bono et al., 2016): unknown duration (but likely years) at >0.066 mg/m^3	Direct and indirect evidence of elevated reactive oxygen species (ROS), possibly at very low concentrations (e.g., at >0.066 mg/m^3, with a maximum of 0.444 mg/m^3) with prolonged human exposure	**Moderate ↑**
		Animal: mRNA changes indicating increased stress-response proteins: (Andersen et al., 2008) short-term ≥2.46 mg/m^3		
	Low	*Human*: Increased nasal lavage nitrites (Priha et al., 2004): acute (8 hr shift) 0.19 mg/m^3	Data suggest elevated oxidative stress at very low formaldehyde concentrations with acute and short-term exposure.	
		Animal: Increased glutathione peroxidase and/or nonprotein sulfhydryl groups (Cassee et al., 1996b) and (Cassee and Feron, 1994): short-term (3 d) 3.94 and 4.43 mg/m^3, respectively		
	High	*Human*: None		**Moderate**

Table A-73. Summary of changes in the upper respiratory tract (URT) resulting from formaldehyde exposure (continued)

Endpoint	Study-specific findings from "high or medium" or "low" confidence experiments		Summary of evidence (exposure duration)	Conclusion
Nasal Cellular Inflammatory Response		*Animal*: Increased inflammatory response, mostly neutrophils but also mention of lymphocytes and other inflammatory cells (e.g., assumed monocytes, basophils and eosinophils): (Monticello et al., 1989) short-term (1 or 6 wk) 7.38 mg/m^3; "inflammatory cell" infiltration: (Andersen et al., 2008) acute or short-term (1 d–3 wk) 7.38 mg/m^3; mRNA and miRNA changes associated with inflammation in rats and nonhuman primates: (Rager et al., 2014; Rager et al., 2013) short-term (1 or 4 wk, with some miRNA changes reversible with 1 wk recovery) at 2.46 mg/m^3: 35 formaldehyde-responsive transcripts altered in the nose known to be related to immune cells indirectly indicated increases in granulocytes (i.e., eosinophil and neutrophil markers) and lymphocyte changes, and (Andersen et al., 2010): short-term (1 wk, but not ≥4 wk) at ≥12.3 mg/m^3	Cellular infiltration observed by histology, primarily neutrophils, but indirectly supporting other immune cell infiltration, in short-term animal studies at 7.38 mg/m^3. Indirect evidence of increases in granulocytes (and possibly lymphocytes) at 2.46 mg/m^3 with short term exposure.	↑ granulocytes (neutrophils, eosinophils); Note: data on lymphocytes considered *Indeterminate*
	Low	*Human*: N/C in nasal lavage cell counts, but increased total protein: Priha et al. (2004) occupationally exposed (8-hr shift) 0.19 mg/m^3; Allergy-independent increased eosinophils, permeability (albumin index) and total protein in lavage: Pazdrak et al. (1993) acute (2 hr) 0.5 mg/m^3; increased eosinophils, leukocytes, and permeability (albumin index) in lavage: (Krakowiak et al., 1998) acute (2 hr) 0.5 mg/m^3 (reversible); indirect evidence of eosinophil infiltration (increased markers: lysozyme and eosinophil cationic protein), but not neutrophils, at very low levels (Norback et al., 2000): <0.02 mg/m^3; unknown duration (likely months or more) in schools	***Suggestive*** of cellular inflammation, particularly eosinophils, at 0.5 mg/m^3 and indirect markers of eosinophil recruitment at lower levels in humans, following acute exposure; neutrophil inflammation observed at ≥6 mg/m^3 in rats with short-term exposure	
		Animal: Neutrophil inflammation: (Monteiro-Riviere and Popp, 1986) short-term ≥6 mg/m^3		
Altered URT Immunity (inferred from URT infections)	High or Medium	*Human*: Increased frequency and duration of URT infections in symptomatic workers; increased chronic URT inflammation (and decreased function of blood neutrophils, but N/C in leukocyte counts) in exposed workers (Lyapina et al., 2004): chronic (yrs) 0.87 mg/m^3 [Note: recent URT infection was often an exclusion criterion in observational studies focusing on pulmonary function; see Section A.5.3)	Indirect evidence of decreased immune capacity in a single study of chronic human exposure at 0.87 mg/m^3 (note: while altered immunity was observed in an mRNA study,	**Slight** ↑URT infection

Table A-73. Summary of changes in the upper respiratory tract (URT) resulting from formaldehyde exposure (continued)

Endpoint	Study-specific findings from "high or medium" or "low" confidence experiments		Summary of evidence (exposure duration)	Conclusion
		Animal: mRNA ***changes Suggestive*** of altered immune response (Andersen et al., 2010): ≥12.3 mg/m^3 short-term (≥1 wk)	these changes were not necessarily indicative of decreased immune response)	
	Low	*Human*: None	No evidence to evaluate	
		Animal: None		

Specific Evaluation and Summary of URT mucociliary function and cellular proliferation

Studies examining the potential effects of formaldehyde exposure on mucociliary function and cell proliferation were considered for use in identifying potential hazards associated with respiratory tract pathology effects, but were ultimately determined to be most useful as mechanistic evidence describing the potential progression of effects on structures within the URT that might lead to more apical effects (e.g., squamous metaplasia). In contrast to the other mechanistic studies described in this section, these observational human studies and experimental animal studies were individually evaluated according to the criteria laid out for human and animal apical endpoint (i.e., hazard) studies described in Appendix A.5.5, noting that the decisions for the specific endpoints considered in this section can differ when interpretations of the reliability of the methods differed from those of the more apical endpoints . Thus, studies were judged as *high*, *medium*, or *low confidence*, or as "not informative" (i.e., not discussed).

Mucociliary function

Mucociliary function studies in animals, which primarily focused on quantifying mucus flow rate and qualitative descriptions of ciliary beat frequency and viscosity, were limited to a set of studies from one research group examining dissected nasal passages. Studies of exposed humans were similarly limited, with relevant endpoints being evaluated in a prevalence study and an acute, controlled exposure study. Data are sparse, but in general, mucus flow and/or ciliary beat were inhibited by formaldehyde exposure as a function of concentration and, at least in rats, exposure duration. Effects were most pronounced in the anterior nasal regions, with effects progressing towards posterior regions after extended exposure durations in rats (see Tables A-74 to A-75). These functional observations are consistent with histological changes observed in experimental animals, including decreased cilia content in rhesus monkeys after 1 or 6 weeks of exposure to 7.38 mg/m^3 (Monticello et al., 1989) and blebbing of ciliary membranes at formaldehyde concentrations as low as 0.62 mg/m^3, with more overt signs of damage at ≥7.38 mg/m^3, in rats exposed for 1 or 4 days (Monteiro-Riviere and Popp, 1986).

In well-conducted experiments in F344 rats, mucociliary function was generally unaffected after exposure to 0.57 mg/m^3 formaldehyde for <1 to 14 days (Morgan et al., 1986a; Morgan et al., 1986c). Although sporadic, minor changes were notable at 2.46 mg/m^3, including slight increases in mucus flow rate, inhibition of ciliary beat and mucus flow became clearly apparent at the next highest concentrations tested, ≥7.27 mg/m^3. Initial increases in mucociliary activity at somewhat lower level formaldehyde concentrations were also apparent immediately after in vitro exposure, including increases in ciliary activity at 1.49 mg/m^3 in ex vivo frog palates and at 1.0 mg/m^3 in dissociated human nasal epithelial cells (Wang et al., 2014b; Morgan et al., 1984), with observations of mucostasis and ciliastasis at ≥5.36 mg/m^3 in frog palates and decreased cilia beating frequency in human epithelial cells at ≥3.46 mg/m^3 (Wang et al., 2014b; Schafer et al., 1999; Morgan et al., 1984); however, these in vitro studies are interpreted with low confidence. Two studies in humans

reported consistent effects, with decreased mucus flow at ≥0.3 mg/m^3 after exposure for several hours, and pathological changes in mucociliary clearance in workers exposed to mean formaldehyde levels of 0.25–0.26 mg/m^3 for several years (Holmström and Wilhelmsson, 1988; Andersen and Molhave, 1983).

In rats, impaired function was most frequent in the dorsal and medial maxilloturbinate, the lateral wall, and portions of the nasoturbinate (Morgan et al., 1986a; Morgan et al., 1986c). This is consistent with the locations of epithelial lesions, which correlate with areas of inhibited ciliary function (Morgan et al., 1986c). Similarly, mucus flow was inhibited in the anterior nose of exposed human volunteers (Andersen and Molhave, 1983). However, whereas mucociliary function was affected with increasing severity with increasing exposure duration over several days in rats (Morgan et al., 1986c), effects on mucus flow rate did not vary with exposure durations of up to several hours in human volunteers (Andersen and Molhave, 1983). Seemingly consistent with this finding, mucociliary function in rat nasal passages was reported to recover considerably within 1 hour after 90 minutes of exposure to 18.5 mg/m^3 (Morgan et al., 1986a); however, less recovery occurred after exposure for 6 hours (Morgan et al., 1986a), and little or no recovery was observable 18 hours after exposure for multiple days at similar concentrations (Morgan et al., 1986c). These data suggest that the initial changes observed in response to exposure may vary somewhat from the functional changes induced by sustained formaldehyde exposure.

Overall, mucociliary function is affected in a concentration-dependent manner shortly after formaldehyde inhalation, and this impaired function can be persistent, at least when exposure exceeds several hours, as indicated by studies in F344 rats and exposed workers. In rats, impaired function worsens with increasing exposure duration, although durations longer than 2 weeks have not been tested.

Table A-74. Mucociliary function studies in experimental animals

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td colspan="2">Rats</td></tr>
<tr><td colspan="2">High confidence</td></tr>
<tr><td>Morgan et al. (1986a)
Fischer 344 rats; male; 3–8/exposed groups and 9/control group.
Exposure: Rats were exposed to FA in dynamic head-only chambers for 10, 20, 45, or 90 min or 6 hrs with or without a 1-hr recovery period.
Test article: Paraformaldehyde.
Actual concentrations were within 5% of nominal concentrations of 0, 2.5, or 18.5 mg/m^3.[1]
Mucociliary function (i.e., mucus flow pattern, mucus flow rate, and ciliary activity) evaluated by using dissected nasal mucosa that included the nasal septum and lateral wall.</td><td>Changes in mucociliary function
<table><tr><th>Group</th><th>Observations</th></tr><tr><td>Controls</td><td>Mean mucus flow rates for nasal septum were slower (0.91–1.2 mm/min) compared to rates on the lateral wall (3.61–8.15 mm/min); lateral wall mucus flow by region (slowest to fastest): anterior, midregions, posterior</td></tr><tr><td>18.5 mg/m^3 (no recovery period)</td><td>Ciliastasis and mucostasis observed in specific regions of nose with discernible differences between recovery and nonrecovery groups; ciliastasis increased progressively with duration of exposure and was observed on anterior and ventral septum, anterio-medial and dorsal maxilloturbinate, and lateral wall and lateral</td></tr></table></td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td>Figure 2 from Morgan et al. (1986b) depicting areas of rat nasal passages used to determine flow rate on nasal septum and lateral wall.

Main limitations: No major limitations</td><td>
<table>
<tr><td></td><td>nasoturbinate; distribution of mucostasis exhibited greater variation within exposure groups compared to ciliastasis; mucostasis exhibited similar site specificity as ciliastasis but with greater coverage than ciliastasis (<1 to several mm posterior to regions of ciliastasis); mucus flow observed over areas of ciliastasis in anterio-medial and anterio-dorsal maxilloturbinate, anterior lateral wall, and anterior septum; mean mucus flow rates reduced in areas of nasal septum and lateral wall with intact mucociliary function</td></tr>
<tr><td>18.5 mg/m^3 (90-min or 6-hr exposure with 1-hr recovery period)</td><td>90-min group: recovery characterized to be almost complete, ciliastasis confined to small regions of anterio-ventral septum, anterio-medial maxilloturbinate, anterio-lateral nasoturbinate, and adjacent lateral wall; extent of ciliastasis similar to 18.5 mg/m^3, 20-min group
6-hr group: recovery characterized as considerable but incomplete, especially in posterior regions of nose; reduced mucus flow rates compared to equivalent regions in control rats</td></tr>
<tr><td>2.5 mg/m^3</td><td>No evidence of impaired mucociliary function</td></tr>
</table>
</td></tr>
<tr><td>Morgan et al. (1986c) (Fischer 344 rats; male; 6 exposed and 12 controls (n=6) morning, n=6 afternoon)/group.
Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for 1, 2, 4, 9, or 14 d. Exposure was followed by an 18-hr recovery period for some groups.
Test article: Paraformaldehyde.
Actual concentrations were 0, 0.57 (0.5−0.6; range), 2.46 (2.4−2.7), 7.27 (7.0−7.5), and 17.7 (15.0−18.5) mg/m^3.[1]
Mucociliary function and mucus flow rate evaluated by using dissected nasal mucosa within 20 min after death.
Histopathologic evaluation of the respiratory tract included transverse sections of the nasal mucosa tissues used in the evaluation of mucociliary function.

Figure 1 from Morgan et al. (1986b) depicting rat nasal passages opened near the midline. Septum was removed to reveal turbinates. Arrows indicate direction of mucus flow, and numbers represent areas assessed for mucus flow rate. Inset represents lateral aspect of nasoturbinate showing lateral scroll.

Main limitations: No major limitations</td><td>
Changes in mucociliary function
<table>
<tr><th>Group</th><th>Observations (truncated from original article)</th></tr>
<tr><td>Controls</td><td>Mucociliary apparatus functioned for 20−60 min after death; minimal inter-animal variation in mucus flow rate</td></tr>
<tr><td>General observations for exposed groups</td><td>Concentration- and duration-related defects included cessation or severe slowing of mucus flow (mucostasis), loss of ciliary function (ciliastasis), or alterations in mucus flow patterns; minimal inter-animal variation; mucostasis observed to generally be more extensive than ciliastasis, mucus was found flowing over areas of inactivated cilia</td></tr>
<tr><td>17.7 mg/m^3</td><td>Duration-dependent mucostasis most frequently observed on dorsal and medial aspects of maxilloturbinate, lateral aspect of nasoturbinate (especially lateral scroll), lateral ridge, and lateral wall; little or no recovery 18 hrs after exposure</td></tr>
<tr><td>7.27 mg/m^3</td><td>Changes were much less extensive as those in 17.7 mg/m^3 group</td></tr>
<tr><td>2.46 mg/m^3</td><td>Changes were characterized as minimal or absent; localized inhibition of ciliary activity for few animals was observed on ventral margin of nasoturbinates with 9 days of exposure</td></tr>
<tr><td>0.57 mg/m^3</td><td>No inhibition of mucociliary function observed</td></tr>
</table>
Changes in mucus flow rate
<table>
<tr><th>Group</th><th>Observations</th></tr>
<tr><td>Controls</td><td>No significant differences observed between morning and afternoon groups, combined for statistical analysis with exposed groups</td></tr>
<tr><td>General observations</td><td>Mucus flow rates found to be characteristic of specific regions of the nose and observed to be slowest on anteromedial naso-and</td></tr>
</table>
</td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td></td><td>
<table>
<tr><td></td><td>maxilloturbinates and anterior margin of ethmoid turbinate, fastest on lateral wall, and intermediate on other regions</td></tr>
<tr><td>17.7 mg/m^3</td><td>Reduction of mean mucus flow rate without histologic changes observed on ventromedial surface of nasoturbinate (area 1) after 1 d of exposure, with more pronounced and statistically significant reductions after 9 d of exposure even with 18 hrs of recovery</td></tr>
<tr><td>7.27 mg/m^3</td><td>No consistent changes in mucus flow rate observed except in areas with mucostasis</td></tr>
<tr><td>2.46 mg/m^3</td><td>No reduction in mucus flow rate observed; nonstatistically significant increases in mean mucus flow rates observed on posteromedial aspect of nasoturbinate (area 10)</td></tr>
<tr><td>0.57 mg/m^3</td><td>No reductions in mucus flow rate observed; statistically significant increases in mean mucus flow rate observed in areas 6 and 9 after 4 d of exposure but not after 9 d of exposure</td></tr>
</table>
</td></tr>
<tr><td colspan="2">Frogs</td></tr>
<tr><td colspan="2">Low confidence</td></tr>
<tr><td>Morgan et al. (1984) Leopard frogs; male; 6/group.
Exposure: Frog palates were exposed to FA in an ex vivo chamber for up to 30 min after a 5-min equilibration period.
Test article: Paraformaldehyde.
Actual concentrations were within 20% of nominal values and are reported for each endpoint in the Results column.[1]
Mucociliary function (i.e., mucus flow and ciliary activity) evaluated by using dissected frog palates.

Main Limitations: ex vivo, acute exposure; nonmamalian model</td><td>
<table>
<tr><th>Group (± SE)</th><th>Initial response[a] to exposure[b]</th><th>Mucus stasis[b] (min ± SE)</th><th>Ciliastasis[b] (min ± SE)</th></tr>
<tr><td>11.8 (±0.37) mg/m^3</td><td>6/6</td><td>6/6 (1.93±0.13)</td><td>6/6 (3.47±0.44)</td></tr>
<tr><td>5.36 (±0.36) mg/m^3</td><td>6/6</td><td>4/6 (8.14±3.27)[c]</td><td>4/6 (13.6±5.18)[c]</td></tr>
<tr><td>1.69 (±0.10) mg/m^3</td><td>6/6[d]</td><td>0/6</td><td>0/6</td></tr>
<tr><td>0.28 (±0.04) mg/m^3</td><td>0/6</td><td>0/6</td><td>0/6</td></tr>
</table>
[a] Response was increased ciliary activity in the presence or absence of increased mucus flow rate.
[b] Number of cases in which change was observed/number of cases examined.
[c] Values in parentheses indicate time to induce the effect for the four positive cases.
[d] The response was variable and generally very slight in this group.

<table>
<tr><th>Group mg/m^3 (± SE)</th><th>Observations for mucociliary function (truncated from original article)</th></tr>
<tr><td>11.8 (±0.37)</td><td>Increased ciliary activity and mucus flow rate; peak mucus flow rate followed by rapid decline, cessation of flow, beating cilia, and changes to mucus flow; ciliastasis preceded by reduced beat frequency and amplitude</td></tr>
<tr><td>5.36 (±0.36)</td><td>Considerable inter-animal variation observed</td></tr>
<tr><td>1.69 (±0.10)</td><td>Inter-animal variation observed; initial response involved variable increase in mucus flow rate and increased ciliary activity or more frequent surges of increased activity</td></tr>
<tr><td>0.28 (±0.04)</td><td>No apparent effect after 30-min exposure</td></tr>
<tr><td>0</td><td>Very few ciliated cells observed to be actively beating; any ciliary beating occurred in individual or small groups of cells; basal mucus flow rate determined to be 0–4 mm/min</td></tr>
</table>
</td></tr>
</table>

As = anterior septum.

[1]Study authors originally reported FA concentrations in ppm. These values were converted based on 1 ppm = 1.23 mg/m^3, assuming 25°C and 760 mm Hg.

Table A-75. Mucociliary function studies in humans

Study and design	Exposure	Results
Medium Confidence		
Andersen and Molhave (1983) *Denmark* *Controlled Human Exposure Study* **Participants:** 16 healthy students, 5 females and 11 males. Mean age: 23 yrs; range 20−33 yrs. 31% smokers with one heavy smoker having >20 cigarettes per day. None had past formaldehyde exposure and all had healthy upper airways. All were habitually nasal breathers with no history of chronic or recent acute respiratory disease. **Methods:** Three identical sets of subject measurements taken each day, first during control period, second after 2−3 hrs of exposure and third after 4−5 hrs of exposure. Nasal mucociliary flow measurements in slits 1−2 are most anterior and slits 5–6 are most posterior part of the ciliated nose. ANOVA significance at 5%. **Main limitations:** short exposure duration; note: internal control	A 5-hr exposure study. Subjects assigned to four groups, each group undergoing four different exposures over 4 consecutive days. Levels were 0.3, 0.5, 1.0 and 2.0 mg/m^3 formaldehyde with order decided by latin square design. Each day began with 2 hr control period using clean air at 23 ± 0.5° C, 50 ± 5 % humidity, air velocity 10 ± 3cm/s and air supply rate of 500 m^3/h. Control air comprised of outdoor air filtered through absolute and charcoal filters. Following control period, formaldehyde was added to air, reaching steady state concentration after one hour. Formaldehyde generated by passing air through an 80°C oven containing paraformaldehyde. Variation monitored, ranging within ±20% from the target values.	A statistically significant decrease in mucus flow rate occurred in the anterior two-thirds portion of the ciliated nose (slits 1–4). Mucus flow rate shown to decrease with increasing formaldehyde concentrations starting at 0.3 mg/m^3 and then leveling off after 0.5 mg/m^3. Flow rate decreases did not fluctuate with time of exposure.
Low Confidence		
Holmström and Wilhelmsson (1988) *Sweden* *Prevalence Study* **Population:** Two exposed groups 170 total; 70 formaldehyde production workers, Mean age 36.9 years, 87% male, mean duration employment 10.4 yr. 100 workers exposed to wood dust and formaldehyde at five furniture factories. Mean age 40.5 years, 93% male, mean duration employment 16.6 yr. Referent: 36	Personal sampling in breathing zone for 1−2 hours in 1985. Total dust and respirable dust also measured. Previous measurements 1979-1984 in chemical company combined with 1985 values to estimate average annual values for each participant. Only 1985 values available for wood factories. Formaldehyde concentration: Chemical plant: 0.05−0.5 mg/m^3, mean 0.26 [SD 0.17 mg/m^3]. Furniture factory: 0.2-0.3 mg/m^3, mean 0.25 [SD 0.05 mg/m^3].	Mucociliary clearance is defined to be pathological if transit time is > 20 minutes for one or both spots. In formaldehyde only group, 20% of subjects (14/69, p <0.05 compared to referent) had clearance times > 20 minutes compared to 15% of the formaldehyde-dust group (14/95) and 3% of the referent group (1/36). Formaldehyde-only nasal specimens had higher mean score of 2.16 (range 0−4) (p <0.05) while

Study and design	Exposure	Results
persons from local government in the same village as the furniture workers, with no history of occupational exposure to formaldehyde or wood dust. Mean age 39.8 yrs, 56% male, mean duration employment 11.4 yr. **Methods:** Pretesting questionnaire, Mucociliary activity tested using green dye spotted on both inferior turbinates 1 cm posterior to the anterior border of the turbinate. Measured transit time of spot to rhinopharynx. Chi-square tests or 2-tailed *t*-test for group comparisons. **Main limitations:** poor matching of referent group (i.e., different occupation type; lower proportion of males); inclusion of only current workers and long duration of employment raises possibility of healthy worker effect due to irritation effects; crude measure.	Referent mean 0.09 mg/m^3 (based on 4 measurements in 4 seasons).	formaldehyde-dust group had mean score 2.07 (range 0–6) (p >0.05). Referent group score was 1.56 (range 0–4). Combining formaldehyde-only and formaldehyde-dust group mean score 2.11 (p <0.05). No correlation observed between smoking habits and biopsy score, nor was a correlation found between the duration of exposure and any histological changes

Cellular proliferation

A number of quantitative cellular proliferation studies have been carried out in experimental animals, primarily in rats. While these experiments provide more robust quantification of changes in cell number compared to histological determinations of tissue hyperplasia, the data provided by these approaches are limited to active proliferation and do not directly inform cumulative proliferative responses. For example, the most common approaches involve in vivo administration of either bromodeoxyuridine (BrdU, a thymidine analog) or tritiated thymidine ([^{3}H]-thymidine), both of which label newly-synthesized DNA in dividing cells. When either of these are administered during the last 1–3 days of an exposure (nearly all of the studies followed a similar protocol), these experiments would only be able to measure the proliferation actively occurring during the 1–3 days at the end of the exposure; they would provide no information on proliferation induced earlier during the exposure period, or on adaptive changes to proliferative responses that might have resulted from those initial exposure effects. Despite this limitation, these studies still provide useful information on the magnitude of acute proliferation induced at different concentrations and following different durations of formaldehyde exposure. In addition, in some studies, histopathology was assessed along with cell proliferation, which may inform potential correlations between cellular proliferation and apical tissue pathology endpoints.

The studies generally assessed cell proliferation in the anterior part of the nasal cavity, focusing on discrete regions (i.e., cross section levels) of the epithelium, with a few studies extending their investigation beyond the nasal cavity to include the trachea, larynx, and carina. There were notable differences in methodology across studies, including the use of different DNA synthesis-labeling agents (i.e., BrdU, [^{3}H] thymidine, ^{14}C), different durations of labeling (i.e., 2 hours to 3 ddays), and different measures of proliferation (i.e., cell turnover; ^{14}C incorporation; labeling index [LI]: the ratio of labeled cells to total counted cells; unit length labeling index [ULLI]: the ratio of labeled cells per mm of basement membrane). While these methodological differences complicate direct comparisons across studies, increases in cell proliferation were in general consistently observed across several rat strains, with supportive findings in smaller databases of mice and monkey studies. Proliferation responses, at least in the anterior nasal cavity of exposed rats, were concentration-dependent, while in most studies the response magnitude remained relatively constant across exposure duration (i.e., acute proliferation responses were not notably larger after longer exposure at similar concentrations; see Figure A-33); the only study to test proliferation beyond 13 weeks of exposure suggested that response magnitude may actually begin to decrease in most nasal regions after chronic exposure (Monticello et al., 1996).

As illustrated in Figure A-33, after ≤1 week, 1–6 weeks, or ≥12 weeks of exposure, proliferation in the nasal epithelium was increased in a concentration-dependent manner in F344 rats, and from a more limited set of studies, in Wistar rats. Proliferation was also shown to increase in single studies of rhesus monkeys (after exposure for either 1 or 6 weeks to 7.38 mg/m^3 formaldehyde; (Monticello et al., 1989)) and B6C3F1 mice (after exposure for 1 to 5 days at approximately 18.45 mg/m^3 formaldehyde; (Chang et al., 1983; Swenberg et al., 1983b)). Interestingly, as with other respiratory tract effects, mice might be less sensitive to changes in cellular proliferation, although the data relevant to this interpretation are sparse. Specifically, proliferation in the epithelium lining nasal associated lymphoid tissue (NALT) was observed in F344 rats, but not in B6C3F1 mice, even at concentrations as high as 18.4 mg/m^3 (Kuper et al., 2011). This potential difference could reflect the differential sensitivity to reflex bradypnea across species (see Section A.3). In rats, although the data were variable across studies, particularly in Wistar rats exposed for ≤ 1 week (Cassee et al., 1996b; Cassee and Feron, 1994; Reuzel et al., 1990; Wilmer et al., 1989; Zwart et al., 1988; Woutersen et al., 1987), the levels of cell proliferation in regions such as the anterior lateral meatus were typically 1.5- to 25-fold greater than control levels after exposure to ≥ ≈12 mg/m^3 formaldehyde, regardless of exposure duration. While levels were similarly increased at ≈6–7.5 mg/m^3 after exposure durations ≤ 13 weeks, the only study to evaluate longer exposures observed less robust increases in proliferation after chronic exposure, as compared to proliferation levels after 3 months of exposure (Monticello et al., 1996). The results across studies were less consistent at formaldehyde concentrations below 4 mg/m^3, with several studies at 2.5–3.67 mg/m^3 indicating that proliferation tended to increase in some nasal regions

after ≥12 weeks (Andersen et al., 2010; Meng et al., 2010; Zwart et al., 1988)[19] and others suggesting elevations in proliferation at concentrations ranging from 1.24–3.69 mg/m^3 with exposure ≤ 1 week (Roemer et al., 1993; Reuzel et al., 1990; Zwart et al., 1988), although not all comparisons in all regions evaluated were statistically significant. Changes at these concentrations were not observed in several other studies of similar exposure duration, or in any studies examining 1–6 weeks of exposure. Increases in proliferation were typically not observed at formaldehyde concentrations below 1.23 mg/m^3, although some weak induction was noted in a few studies.

Proliferation generally exhibited a decreasing anterior to posterior gradient and correlated with sites of respiratory tract pathology. For example, after adjusting for the number of animals with accurate tumor localization and including target cell population size in the comparison, increased cell proliferation was correlated (R^2 = 0.88) with the incidence of squamous cell carcinoma; however, cell proliferation alone (i.e., without considering target cell population size) was not as well correlated (Monticello et al.), suggesting that some minimal cell population size may be important for tumor formation. Cell proliferation has also been shown to be correlated with hyperplasia and squamous metaplasia; nasal lesions indicative of cytotoxicity such as cell degeneration, necrosis, or erosion and/or inflammation (Speit et al., 2011b; Andersen et al., 2010; Andersen et al., 2008; Monticello et al., 1991). Although most studies demonstrated proliferation in anterior regions of the nasal cavity, primarily examining sections at cross level 2 (variably including anterior and/or medial portions of structures such as the lateral meatus, maxilloturbinate, and nasoturbinate), some studies demonstrated formaldehyde-induced changes in more posterior regions, including regions outside of the URT. For example, exposure of groups (n = 3) of rhesus monkeys to 7.36 mg/m^3 for 1 or 6 weeks resulted in increased proliferation along with slight histological changes (e.g., inflammation, hyperplasia, and metaplasia) in both the nasal cavity and extranasal regions including the larynx, trachea, and carina, but not the bronchioles (Monticello et al., 1989). In F344 rats, increased proliferation was observed in the nasopharynx at ≥12.3 mg/m^3 (with slight increases at 2.48 mg/m^3) after 4 weeks of exposure (Speit et al., 2011b). Increased proliferation in the trachea and lung was observed in SD rats following 1 or 3 days of exposure to 24.6 mg/m^3, with mixed findings at lower concentrations, including increased proliferation in the trachea at 2.5mg/m^3 after 1 day of exposure, but decreased proliferation in the trachea with 3 days of exposure at 2.5–7.4 mg/m^3 (Roemer et al., 1993).

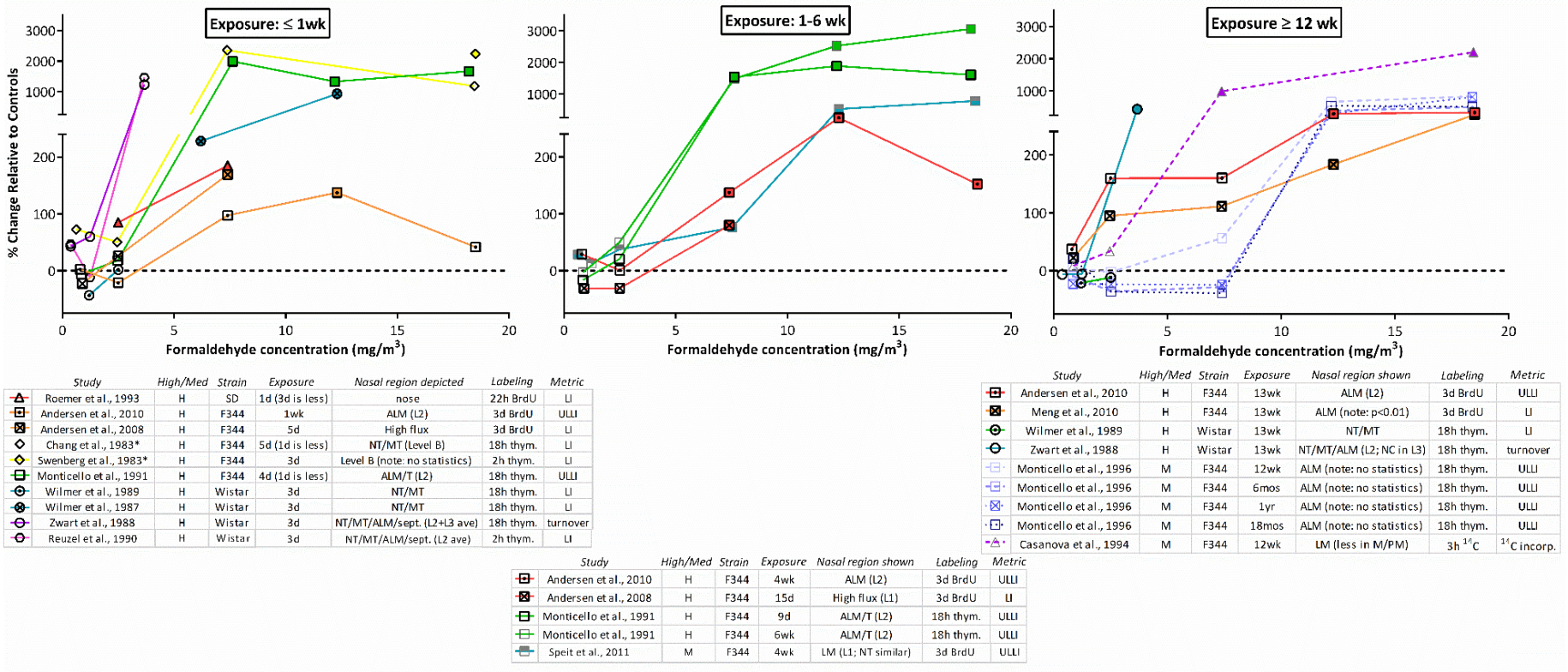
These latter data highlight the complicated nature of the association between formaldehyde exposure duration and cellular proliferation. While, generally, proliferation appears to be sustained at similar levels across exposure durations ranging from 1 day to 13 weeks (see Figure A-33), some studies reported differences in the magnitude of effects in specific regions of the respiratory tract tissue after different exposure durations. In studies of F344 and Wistar rats exposed to a wide

[19] These data from Meng et al. are revisited in the context of uncertainty and variability in the dose-response for cell replication in B.2.2.

range of formaldehyde concentrations (0.37–18.5 mg/m^3), proliferation induced by formaldehyde exposure was typically not increased with longer exposure duration (in some instances, it was slightly decreased, but statistical comparisons were not performed) in various anterior nasal sections (approximately levels I-III), including comparisons of 3 days to 10 days (Chang et al., 1983; Swenberg et al., 1983b), 5 days to 15 days (Andersen et al., 2008), and 4 days to 6 weeks (Monticello et al., 1991) in F344 rats (note: response magnitude increased from 1 to 4 days in the latter study) and comparisons of 3 days to 4 weeks (Wilmer et al., 1987) and 3 days to 13 weeks in Wistar rats (Zwart et al., 1988). In several of these studies, the data suggest that formaldehyde concentration had a much greater impact on proliferation than exposure duration, although the relative contributions of concentration versus duration could not be accurately defined (Wilmer et al., 1989, 1987; Chang et al., 1983; Swenberg et al., 1983b). Somewhat complicating this, an increasing magnitude of proliferation at the same formaldehyde concentration was observed in anterior nasal regions of F344 rats exposed to 7.4–18.5 mg/m^3 for 13 weeks, as compared to 1 or 4 weeks (Andersen et al., 2010), or for 5 days, as compared to 1 day (Chang et al., 1983), although an increase was not observed in B6C3F1 mice in the latter study. Similarly, in a study of rhesus monkeys, there was a noted exposure duration-dependent increase in proliferation in more posterior regions (approximately nasal section levels III-V as well as regions posterior to the nasal cavity) at 7.4 mg/m^3 from 1 to 6 weeks of exposure (Monticello et al., 1989). Interestingly, while duration-dependent increases in proliferation were observed in anterior nasal regions of F344 rats exposed to 0.86–18.5 mg/m^3 for 1–13 weeks, cell proliferation was greatest at 4 weeks, as compared to 1 or 13 weeks, when examining central and posterior portions (levels 2–3) of the nasal cavity (Meng et al., 2010). Finally, as previously mentioned and of particular interest, are the results of Monticello et al. (1996) in F344 rats exposed to 0.85–18.4 mg/m^3 formaldehyde. The authors observed decreases in proliferation when comparing 3 months of exposure with longer durations up to 18 months within most of the nasal regions examined, including the lateral meatus, the anterior and posterior mid-septum, and medial maxilloturbinate; however, the opposite finding (i.e., duration-dependent increases in proliferation) was observed in the anterior dorsal septum (Monticello et al., 1996). Overall, the pattern across studies is mixed but indicates region-specific differences in the impact of exposure duration on proliferation.

A large number of well-conducted studies have evaluated acute cellular proliferation after exposure to a wide range of formaldehyde concentrations for durations ranging from 1 day to 18 months. The data were variable across studies. This variability is assumed to result, at least in part, from methodological factors that include the selection and preparation of tissue for analysis, the composition and administration protocol of the labeling agent used to indicate proliferation, when the proliferation counts were made (e.g., age of the animal), and the units used to express proliferation data (e.g., LI versus ULLI) (Monticello and Morgan, 1997; Goldsworthy et al., 1993; Monticello et al., 1993; Goldsworthy et al., 1991). Despite this methodological variability, cell proliferation was consistently increased in response to formaldehyde exposure in anterior portions

of the rat, mouse, and monkey nasal cavity, with studies in rats demonstrating a prominent role for formaldehyde concentration. While some studies in rats and monkeys demonstrated a role for exposure duration in cell proliferation within specific regions of the respiratory tract, acute proliferation in most nasal regions generally remained constant regardless of exposure duration. The variability in the labeling index data in Monticello et al. (1996; 1991) is extensively characterized in B.2.2 "Characterization of uncertainty and variability in cell replication rates."



Study	High/Med	Strain	Exposure	Nasal region depicted	Labeling	Metric
Roemer et al., 1993	H	SD	1d (3d is less)	nose	22h BrdU	LI
Andersen et al., 2010	H	F344	1wk	ALM (L2)	3d BrdU	ULLI
Andersen et al., 2008	H	F344	5d	High flux	3d BrdU	LI
Chang et al., 1983*	H	F344	5d (1d is less)	NT/MT (Level B)	18h thym.	LI
Swenberg et al., 1983*	H	F344	3d	Level B (note: no statistics)	2h thym.	LI
Monticello et al., 1991	H	F344	4d (1d is less)	ALM/T (L2)	18h thym.	ULLI
Wilmer et al., 1989	H	Wistar	3d	NT/MT	18h thym.	LI
Wilmer et al., 1987	H	Wistar	3d	NT/MT	18h thym.	LI
Zwart et al., 1988	H	Wistar	3d	NT/MT/ALM/sept. (L2+L3 ave)	18h thym.	turnover
Reuzel et al., 1990	H	Wistar	3d	NT/MT/ALM/sept. (L2 ave)	2h thym.	LI

Study	High/Med	Strain	Exposure	Nasal region shown	Labeling	Metric
Andersen et al., 2010	H	F344	4wk	ALM (L2)	3d BrdU	ULLI
Andersen et al., 2008	H	F344	15d	High flux (L1)	3d BrdU	LI
Monticello et al., 1991	H	F344	9d	ALM/T (L2)	18h thym.	ULLI
Monticello et al., 1991	H	F344	6wk	ALM/T (L2)	18h thym.	ULLI
Speit et al., 2011	M	F344	4wk	LM (L1; NT similar)	3d BrdU	ULLI

Study	High/Med	Strain	Exposure	Nasal region shown	Labeling	Metric
Andersen et al., 2010	H	F344	13wk	ALM (L2)	3d BrdU	ULLI
Meng et al., 2010	H	F344	13wk	ALM (note: p<0.01)	3d BrdU	LI
Wilmer et al., 1989	H	Wistar	13wk	NT/MT	18h thym.	LI
Zwart et al., 1988	H	Wistar	13wk	NT/MT/ALM (L2; NC in L3)	18h thym.	turnover
Monticello et al., 1996	M	F344	12wk	ALM (note: no statistics)	18h thym.	ULLI
Monticello et al., 1996	M	F344	6mos	ALM (note: no statistics)	18h thym.	ULLI
Monticello et al., 1996	M	F344	1yr	ALM (note: no statistics)	18h thym.	ULLI
Monticello et al., 1996	M	F344	18mos	ALM (note: no statistics)	18h thym.	ULLI
Casanova et al., 1994	M	F344	12wk	LM (less in M/PM)	3h ^{1c}C	^{1c}C incorp.

Figure A-33. Nasal cell proliferation in rats exposed to formaldehyde. Summary of rat studies of nasal cell proliferation (as % change relative to controls) following different durations of formaldehyde exposure, specifically ≤1 week (left panel), 1–6 weeks (center panel), or ≥ 12 weeks (right panel). T he tables below each panel summarize the studies, study confidence determinations (only high and medium confidence studies are shown), exposure durations, nasal regions depicted, cell labeling methods used, and the method of data reporting for each corresponding panel. Note: solid symbols indicate statistical significance, as identified by the study authors. High confidence studies are indicated by bolder symbols and with solid, rather than dashed, connecting lines. Data at different timepoints from the same study are indicated by use of the same line colors and general symbol shapes. See Tables A-76 and A-77 for additional details.

Table A-76. Subchronic or chronic exposure cell proliferation studies in experimental animals

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td colspan="2">Rats</td></tr>
<tr><td colspan="2">High confidence</td></tr>
<tr><td>Andersen et al. (2010) Fisher 344; male; 8/group.
Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for 1, 4, or 13 wks. Rats sacrificed immediately after last exposure.
Test article: Paraformaldehyde.
Actual concentrations reported in the Results column. Target concentrations were 0, 0.8, 2.5, 7.4, 12.3, and 18.5 mg/m^3.[1]

Cell proliferation studies conducted with surgical implantation of BrdU-containing pumps (3 days prior to sacrifice) and determining labeling index at levels I (highest FA flux near nose tip), II (anterior lateral meatus, anterior mid-septum, medial aspect of maxilloturbinate), and III (posterior lateral meatus, posterior mid-septum). Cell proliferation at each site reported as number of labeled cells per total cells (i.e., LI) and as the number of labeled cells per length (i.e., mm) of basement membrane (i.e., ULLI).

Supplemental 4A from Andersen et al. (2010) depicting a schematic illustration of the nasal cavity levels used for cell proliferation studies.</td><td>Nasal Epithelium ULLI
<table><tr><td></td><td colspan="3">Formaldehyde (mg/m^3)</td></tr><tr><td>Site</td><td>0</td><td>0.8</td><td>2.5</td></tr><tr><td>High-flux region (HFR)</td><td></td><td></td><td></td></tr><tr><td>1 week</td><td>12.8±3.5[a] (7)[b]</td><td>15.0±12.5 (8)</td><td>13.8±7.0 (8)</td></tr><tr><td>4 weeks</td><td>20.3±4.1 (7)</td><td>17.8±3.8 (8)</td><td>18.5±4.6 (5)</td></tr><tr><td>13 weeks</td><td>21.9±20.3 (3)</td><td>12.2±10.3 (3)</td><td>29.1±32.7 (6)</td></tr><tr><td>Anterior lateral meatus (ALM)</td><td></td><td></td><td></td></tr><tr><td>1 week</td><td>31.9±26.3 (8)</td><td>32.6±30.2 (8)</td><td>25.1±26.1 (8)</td></tr><tr><td>4 weeks</td><td>26.6±17.1 (8)</td><td>34.3±21.3 (8)</td><td>26.7±7.9 (8)</td></tr><tr><td>13 weeks</td><td>21.7±15.1 (8)</td><td>29.7±24.6 (8)</td><td>56.3±33.3 (8)</td></tr></table>[a]Mean ULLI±SD; [b]Number of animals examined.

Nasal Epithelium ULLI (continued)
<table><tr><td></td><td colspan="4">Formaldehyde (mg/m^3)</td></tr><tr><td>Site</td><td>0</td><td>7.4</td><td>12.3</td><td>18.5</td></tr><tr><td>High flux region (HFR)</td><td></td><td></td><td></td><td></td></tr><tr><td>1 week</td><td>12.8±3.5[a] (7)[b]</td><td>25.2±13.3 (8)</td><td>36.1±14.3[c] (8)</td><td>25.3±17.5 (7)</td></tr><tr><td>4 weeks</td><td>20.3±4.1 (7)</td><td>40.9±24.9 (5)</td><td>69.2±17.7[c] (6)</td><td>63.6±26.1[c] (8)</td></tr><tr><td>13 weeks</td><td>21.9±20.3 (3)</td><td>17.4 (1)</td><td>58.3±27.8 (5)</td><td>110.2±46.0[c] (7)</td></tr><tr><td>Anterior lateral meatus (ALM)</td><td></td><td></td><td></td><td></td></tr><tr><td>1 week</td><td>31.9±26.3 (8)</td><td>62.9±50.3 (8)</td><td>75.7±31.1[d] (8)</td><td>45.1±25.7 (8)</td></tr><tr><td>4 weeks</td><td>26.6±17.1 (8)</td><td>63.1±21.6[c] (8)</td><td>90.7±17.6[c] (8)</td><td>67.0±10.5[c] (8)</td></tr><tr><td>13 weeks</td><td>21.7±15.1 (8)</td><td>56.4±17.2 (8)</td><td>83.3±33.3[c] (8)</td><td>91.8±33.1[c] (8)</td></tr></table>[a]Mean ULLI±SD; [b]Number of animals examined; [d]$p<0.01$; [e]$p<0.05$.</td></tr>
<tr><td>Meng et al. (2010)
Fischer 344; males; 8/group.
Exposure: Rats were exposed to FA in dynamic chambers (not otherwise specified) 6 hrs/d, 5 d/wk for 1, 4, or 13 wks.
Test article: Paraformaldehyde.
Actual concentrations were not reported. Target concentrations were 0, 0.86, 2.46, 7.38, 12.3, and 18.5 mg/m^3.</td><td>Dose-dependent increases in cell proliferation of nasal epithelium at 1, 4, and 13 wks of exposure.

Cell proliferation had a decreasing anterior to posterior gradient.

Duration-dependent increases in cell proliferation at the anterior portion of nasal cavity.</td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td>Cell proliferation studies conducted with surgical implantation of BrdU-containing pumps (3 d prior to sacrifice) and determining labeling index in the anterior lateral meatus (lateral wall) for both sides of the nose. Cell proliferation data reported as percentage of BrdU-labeled cells among the total number of labeled and unlabeled cells.</td><td>Cell proliferation greatest in the central and posterior regions of the nose following 4 weeks of exposure.

FA (mg/m^3) | % BrdU-labeled cells after 13 wk
0 | 18
0.86 | 22
2.46 | 35
7.38 | 38
12.3 | 51[a]
18.5 | 64[a]
[a]p <0.01, compared to control group</td></tr>
<tr><td>Wilmer et al. (1989)
Wistar rats; male; 25/group.
Exposure: Rats were exposed to FA in dynamic horizontally placed glass cylinders (with sampling ports at the inlet and outlet) either continuously for 8 hrs/d, 5 d/wk for 13 wks or intermittently 8 hrs/d (successive periods of 0.5 hr of exposure and 0.5 hr of nonexposure), 5 d/wk for 13 wks.
Test article: Paraformaldehyde.
Actual concentrations were not determined. Target concentrations were 0, 1.2, or 2.5 mg/m^3 for continuous exposures and 0, 2.5, or 4.9 mg/m^3 for intermittent exposures.[1]
Cell proliferation studies carried out after 3 d or 13 wks of FA exposure with [^{3}H]thymidine labeling (ip injection 18 hrs postexposure) and scoring of the cells lining the nasal (n=1,000) and maxillary (n=1,000) turbinates and the septum (n=3,000).</td><td>Percentage of [^{3}H]thymidine labeled cells in nasal epithelium
Exposure | Exposure x time | % labeled cells After 3 d | After 13 wk
0 mg/m^3 | 0 mg/m^3 hr/d | 0.60 (0.37)[a] | 1.03 (0.26)
1.2 mg/m^3 (continuous) | 9.6 mg/m^3 hr/d | 0.34 (0.10) | 0.81 (0.54)
2.5 mg/m^3 (continuous) | 20 mg/m^3 hr/d | 0.61 (0.28) | 0.91 (0.59)
2.5 mg/m^3 (intermittent) | 10 mg/m^3 hr/d | 0.29 (0.20) | 1.16 (0.59)
4.9 mg/m^3 (intermittent) | 19.6 mg/m^3 hr/d | 0.58 (0.32) | 2.86 (1.80)
[a] SDs shown in parentheses.</td></tr>
<tr><td>Zwart et al. (1988) Wistar rats; male and female; 50/group/sex.
Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/day, 5 d/wk for 13 wks.
Test article: Paraformaldehyde.
Actual concentrations were 0, 0.37 (±0.02), 1.24 (±0.10), and 3.67 (±0.27) mg/m^3.[1]
Cell proliferation studies carried out after 3 d or 13 wks of FA exposure with [^{3}H]thymidine labeling (i.p. injection 18 hrs postexposure) and scoring of the</td><td>Cell proliferation (based on 5 rats/group/sex)
3 days:
Section III − Exposure-related increase in cell turnover for combined data (males and female, p <0.001), with statistically significant differences between males and females (p <0.02).
Section II − Cell turnover statistically significant (p <0.001) in 3.67 mg/m^3 group, no difference in 0.37 and 1.24 mg/m^3 groups compared to controls.
13 weeks:
Section III − Statistically nonsignificant decrease in mean cell turnover for all groups.</td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td>cells lining the nasal and maxillary turbinates (n=1,500), septum (n=2,000), and lateral wall (n=1,500) at Section III. Only cells lining the nasal septum were scored at Section II.</td><td>Section II − Cell turnover statistically significant (p <0.001) in 3.67 mg/m^3 group, no difference in 0.37 and 1.24 mg/m^3 groups compared to controls.
Compared to Section II, cell turnover roughly 10 times greater at Section III.

Data extracted using GrabIt software (mean+SEM converted from log scale):
<table>
<tr><th>mg/m^3</th><th>Level III (3 d)</th><th>Level III (13 wk)</th><th>Level II (3 d)</th><th>Level II (13 wk)</th></tr>
<tr><td>0</td><td>0.517 (0.043)</td><td>0.165 (0.029)</td><td>0.022 (0.005)</td><td>0.041 (0.014)</td></tr>
<tr><td>0.37</td><td>0.541 (0.045)</td><td>0.133 (0.021)</td><td>0.040 (0.008)</td><td>0.038 (0.010)</td></tr>
<tr><td>1.24</td><td>0.872 (0.104)*</td><td>0.141 (0.027)</td><td>0.034 (0.009)</td><td>0.038 (0.005)</td></tr>
<tr><td>3.67</td><td>3.71 (0.442)*</td><td>0.101 (0.027)</td><td>0.435 (0.147)*</td><td>0.214 (0.050)*</td></tr>
</table></td></tr>
<tr><td colspan="2">Medium confidence</td></tr>
<tr><td>Casanova et al. (1994)
Fischer 344; male; 8/group.
Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for 11 wks plus 4 d. On day 5 of week 12, rats were exposed to labeled FA (i.e., H^{14}CHO) in nose-only chambers for 3 hrs.
Test article: Paraformaldehyde.
Actual concentrations were 0, 0.86 (±0.02), 2.52 (±0.05), 7.23 (±0.16), 12.35 (±0.23), 17.86 (±0.37) mg/m^3 for whole body exposures and 0, 0.86 (±0.02), 2.53 (±0.04), 7.39 (±0.15), and 19.4 (±0.4) mg/m^3 for nose-only exposures.[1]

Cell proliferation studies carried out by determining H^{14}CHO incorporation into DNA (i.e., de novo DNA synthesis) via liquid scintillation counting.</td><td>Cell proliferation lateral meatus (LM) versus medial and posterior meatuses (M:PM)[a]
<table>
<tr><th>FA (mg/m^3)[b]</th><th>Observation</th></tr>
<tr><td>0</td><td>NA</td></tr>
<tr><td>0.86</td><td>No difference between LM and M:PM</td></tr>
<tr><td>2.53</td><td>No difference between LM and M:PM</td></tr>
<tr><td>7.39</td><td>Preexposed (PE) rats: significantly greater (p≤0.02) proliferation in LM than M:PM
Naïve (N) rats: greater proliferation in M:PM than LM</td></tr>
<tr><td>19.4</td><td>PE rats: significantly greater (p≤0.02) proliferation in LM than M:PM

N rats: greater proliferation in M:PM than LM</td></tr>
</table>
[a]For whole body exposures to unlabeled FA, rats exposed to 0 mg/m^3 were considered N, whereas rats in the other exposure groups were considered PE; [b]Concentrations represent those used for nose-only exposures with H^{14}CHO.

Cell proliferation preexposed versus naïve rats[a]
<table>
<tr><th>FA (mg/m^3)[b]</th><th>Observation[c]</th></tr>
<tr><td>0</td><td>NA</td></tr>
<tr><td>0.86</td><td>No difference between PE and N</td></tr>
<tr><td>2.53</td><td>No difference between PE and N</td></tr>
<tr><td>7.39</td><td>PE rats: greater (p <0.01) proliferation in LM than in N rats</td></tr>
<tr><td>19.4</td><td>PE rats: greater (p <0.01) proliferation in LM and M:PM than N rats</td></tr>
</table></td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td></td><td>[a]For whole body exposures to unlabeled FA, rats exposed to 0 mg/m^3 were considered N, whereas rats in the other exposure groups were considered PE; [b]Concentrations represent those used for nose-only exposures with H^{14}CHO.
[c]Lateral meatus = L; medial and posterior meatuses = M:PM.

Data extracted using GrabIt software (mean+SEM):
<table>
<tr><th>mg/m^3</th><th>Lateral Meatus (3h)</th><th>Lateral Meatus (12 wk)</th><th>Med/Posterior Meatus (3d)</th><th>Med/Posterior Meatus (12 wk)</th></tr>
<tr><td>0.861</td><td>69.16 (0.0001)</td><td>74.93 (5.76)</td><td>57.63 (5.76)</td><td>63.40 (5.76)</td></tr>
<tr><td>2.46</td><td>80.69 (5.76)</td><td>92.22 (5.76)</td><td>97.98 (0.0001)</td><td>109.5 (5.76)</td></tr>
<tr><td>7.38</td><td>115.3 (5.76)</td><td>749.3 (161.4)*</td><td>201.7 (23.05)</td><td>276.7 (23.05)</td></tr>
<tr><td>18.45</td><td>149.86 (11.53)</td><td>1591 (132.5)*</td><td>334.3 (23.05)</td><td>1002 (103.7)*</td></tr>
</table>
*p<0.05 for 12 wk vs 3 hr exposure</td></tr>
<tr><td>Monticello et al. (1996)
F344 rats; male; 6/group.
Exposure: Rats were exposed to FA in dynamic whole-body chambers to FA 6 hrs/d, 5 d/wk for up to 24 mos with interim sacrifices at 3, 6, 12, and 18 mos.
Test article: Paraformaldehyde.
Actual FA concentrations were 0 (±0.0), 0.85 (±0.06), 2.52 (±0.18), 7.39 (±0.41), 12.2 (±0.54), or 18.4 (±0.98) mg/m^3.[1]
Cell proliferation studies (6 rats/group) conducted with surgical implantation of [methyl-^{3}H]thymidine-containing pumps (5 days prior to interim sacrifice) and determining labeling index at 7 locations in the nasal passages: anterior lateral meatus, posterior lateral meatus, anterior mid-septum, posterior mid-septum, anterior dorsal septum, medial maxilloturbinate, and maxillary sinus (excluding ostium). Cell proliferation data reported as the number of labeled cell profiles per mm of basement membrane (i.e., ULLI).</td><td>
<table>
<tr><th>mg/m^3</th><th>Exposure (mos)</th><th>Anterior lateral meatus</th><th>Posterior lateral meatus</th><th>Anterior mid-septum</th><th>Posterior mid-septum</th><th>Anterior dorsal septum</th></tr>
<tr><td>0</td><td>3</td><td>10.11[a]</td><td>7.69</td><td>6.58[a]</td><td>11.94</td><td>2.14</td></tr>
<tr><td></td><td>6</td><td>11.14</td><td>11.92</td><td>5.73</td><td>27.31</td><td>3.61</td></tr>
<tr><td></td><td>12</td><td>8.28</td><td>7.67</td><td>3.25</td><td>31.31</td><td>8.63</td></tr>
<tr><td></td><td>18</td><td>5.74</td><td>8.99</td><td>4.80</td><td>19.86</td><td>3.80</td></tr>
<tr><td>0.85</td><td>3</td><td>10.53</td><td>7.82</td><td>8.04</td><td>13.28</td><td>1.08</td></tr>
<tr><td></td><td>6</td><td>10.09</td><td>8.15</td><td>3.71</td><td>17.04</td><td>2.20</td></tr>
<tr><td></td><td>12</td><td>6.39</td><td>5.11</td><td>1.72</td><td>13.28</td><td>1.08</td></tr>
<tr><td></td><td>18</td><td>6.89</td><td>6.40</td><td>4.54</td><td>18.31</td><td>4.95</td></tr>
<tr><td>2.52</td><td>3</td><td>9.83</td><td>11.24[b]</td><td>12.74</td><td>13.11[b]</td><td>3.38</td></tr>
<tr><td></td><td>6</td><td>7.14</td><td>9.15</td><td>4.78</td><td>12.07</td><td>2.06</td></tr>
<tr><td></td><td>12</td><td>6.35</td><td>6.19</td><td>2.14</td><td>10.35</td><td>0.92</td></tr>
<tr><td></td><td>18</td><td>3.66</td><td>5.24</td><td>3.02</td><td>7.20</td><td>1.93</td></tr>
<tr><td>7.39</td><td>3</td><td>15.78</td><td>9.65</td><td>4.15</td><td>10.52</td><td>3.55</td></tr>
<tr><td></td><td>6</td><td>7.98</td><td>6.74</td><td>3.52</td><td>7.76</td><td>1.52</td></tr>
<tr><td></td><td>12</td><td>6.24</td><td>5.42</td><td>3.06</td><td>8.76</td><td>2.01</td></tr>
<tr><td></td><td>18</td><td>3.51</td><td>6.47</td><td>3.96</td><td>12.30</td><td>1.96</td></tr>
<tr><td>12.2</td><td>3</td><td>76.79</td><td>15.29</td><td>39.01</td><td>21.43</td><td>5.28</td></tr>
<tr><td></td><td>6</td><td>53.57</td><td>17.97</td><td>28.22</td><td>15.81</td><td>2.64</td></tr>
<tr><td></td><td>12</td><td>32.42</td><td>5.60</td><td>10.29</td><td>6.79</td><td>2.20</td></tr>
<tr><td></td><td>18</td><td>36.28</td><td>19.45</td><td>11.92</td><td>24.44</td><td>3.22</td></tr>
<tr><td>18.4</td><td>3</td><td>93.22</td><td>59.52</td><td>75.71</td><td>51.79</td><td>5.96</td></tr>
<tr><td></td><td>6</td><td>65.89</td><td>44.63</td><td>75.32</td><td>61.52</td><td>26.18</td></tr>
<tr><td></td><td>12</td><td>74.99</td><td>44.73</td><td>51.62</td><td>60.56</td><td>37.52</td></tr>
</table>
</td></tr>
</table>

Reference and study design	Results

	18	34.62	22.34	30.29	37.06	52.98

[a]n=5 or 6; [b]n=4

Exposure (mos)	*mg/m³*	*medial maxilla turbinate*	*maxillary sinus*	*mg/m³*	*medial maxilla turbinate*	*maxillary sinus*
3	0	7.84[a]	8.10	7.39	9.23	ND
6		17.95	ND		10.18	ND
12		7.85	6.31		6.22	12.04
18		5.58	5.95		5.03	9.51
3	0.85	10.33	ND	12.2	89.20	ND
6		9.34	ND		57.83	ND
12		6.79	7.80		43.27	9.15
18		5.08	6.99		42.74	12.12
3	2.52	10.84	3.12	18.4	115.19	10.77[b]
6		10.41	ND		101.97	13.13
12		5.98	7.73		66.64	17.06
18		3.42	8.52		63.11	13.16

[a]n=5 or 6; [b]n=3

* $p < 0.05$ as reported by the study authors, unless otherwise indicated

Table A-77. Short-term exposure cell proliferation studies in experimental animals

Reference and study design	Results
Rats	
High Confidence	

Andersen et al. (2008)

Fischer 344 rats; male; 8/group.

Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for up to 3 wks. Rats sacrificed at end of single 6-hr exposure (Day 1), 18 hrs after single 6-hr exposure (Day 1 recovery), at end of 5 d of exposure (Day 5), at end of 6 d of exposure (Day 6), 18 hrs after 6 d of exposure (Day 6 recovery), and at end of 15 d of exposure (Day 15).

Test article: Paraformaldehyde.

Actual concentrations were determined on a daily basis and reported in the **Results** column. Target concentrations were 0, 0.9, 2.5, 7.4, and 18.5 mg/m³.[1]

Target concentration (mg/m³)	*Actual FA Concentrations[a]*			
	Day 1 (mg/m³)	*Day 5 (mg/m³)*	*Day 6 (mg/m³)*	*Day 15 (mg/m³)*
0	0±0	0±0	0±0	0±0
0.9	0.74±0.23	0.79±0.15	0.75±0.16	0.7±0.11
2.5	2.08±0.46	2.14±0.43	2.26±0.49	2.2±0.31
7.4	5.83±1.73	6.43±0.76	6.00±1.25	6.14±0.97
18.5	17.7±5.7	NA	NA	NA

[a]Daily means ± SD.

Cell proliferation in nasal epithelium[a]

			Formaldehyde (mg/m³)			
Day	Level	Site	Control	0.9	2.5	7.4
5	I	NA	38.6±8.5[b] (13.2±4.6)	36.8±14.7 (10.2±2.8)	65.0±39.8 (16.6±6.0)	155.0±88.9[c] (35.5±14.8)[c]
	II	Alm	6.0±2.5	7.5±1.1	7.3±1.7	29.0±21.9[c]
		As	5.6±3.0	6.0±1.6	6.6±3.5	14.2±10.3[c]

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td>This study also evaluated the effects of a single FA instillation (40 µL, 400 mM per nostril). Data presented here in the Results column are for inhalation exposures.

Cell proliferation studies conducted with surgical implantation of BrdU-containing pumps (3 d prior to sacrifice) and determining labeling index at levels I (front of nose), II (anterior lateral meatus, anterior septum, medial aspect maxilloturbinate), and III (posterior lateral meatus, posterior septum). Cell proliferation determined only for days 5 and 15 and reported as the number of labeled cell profiles per mm of basement membrane (i.e., ULLI).</td><td>
<table>
<tr><td></td><td></td><td>Mam</td><td>6.5±2.1</td><td>6.8±3.1</td><td>9.7±3.8</td><td>35.1±22.0[c]</td></tr>
<tr><td></td><td>III</td><td>Plm</td><td>6.4±3.0</td><td>8.1±2.4</td><td>10.0±4.0</td><td>16.1±6.4[c]</td></tr>
<tr><td></td><td></td><td>Ps</td><td>8.9±3.0</td><td>7.5±3.5</td><td>8.0±5.2</td><td>15.0±11.9[c]</td></tr>
<tr><td rowspan="7">15</td><td>I</td><td>NA</td><td>78.9±54.7
(22.6±17.2)</td><td>55.8±37.3
(15.6±10.5)</td><td>50.8±44.2
(15.6±13.1)</td><td>119.1±38.0
(40.6±11)[c]</td></tr>
<tr><td rowspan="3">II</td><td>Alm</td><td>12.4±12.4</td><td>18.2±11.4</td><td>12.1±7.0</td><td>19.1±8.7</td></tr>
<tr><td>As</td><td>12.0±9.7</td><td>17.6±11.0</td><td>10.0±4.6</td><td>14.1±8.7</td></tr>
<tr><td>Mam</td><td>22.7±23.0</td><td>27.2±18.6</td><td>20.9±20.6</td><td>21.9±16.8</td></tr>
<tr><td rowspan="2">III</td><td>Plm</td><td>11.8±10.0</td><td>12.6±6.3</td><td>11.7±7.6</td><td>13.6±7.2</td></tr>
<tr><td>Ps</td><td>15.9±15.2</td><td>13.0±5.9</td><td>12.5±6.3</td><td>18.3±12.1</td></tr>
</table>
[a]Reported as mean±SD; [b]Data represent ULLI. Data in parenthesis represent LI: (labeled cells/total cells) × 100; [c]p <0.05.
</td></tr>
<tr><td>Cassee et al. (1996b)
Wistar rats; male; 5 to 6/group.
Exposure: Rats were exposed to FA in dynamic nose-only chambers 6 hrs/d for 1 or 3 d. Rats sacrificed immediately after last exposure.
Test article: Paraformaldehyde. Actual concentrations were 0, 1.2, 3.9, and 7.9 mg/m^3.[1]

Cell proliferation studies carried out using deparaffinized standard cross sections of the nose and semi-quantitative proliferating cell nuclear antigen (PCNA) immunostaining. Cell proliferation studies were also conducted with surgical implantation of BrdU-containing pumps (20 hrs prior to sacrifice). Labeling index determined for the entire epithelium of both sides of anterior nasal cavity lining the nasoturbinate, maxilloturbinate, lateral wall, and septum. Cell proliferation at each site reported as number of positive-stained cells per length (i.e., mm) of basement membrane (i.e., ULLI).</td><td>1 d exposure: no treatment-related changes in cell proliferation
<table>
<tr><th>FA (mg/m^3)</th><th>Cell proliferation measured by PCNA after 3 days[a]</th></tr>
<tr><td>1.2</td><td>Levels II and III: no increases in ULLIs</td></tr>
<tr><td>3.9</td><td>Level II: significant increase in ULLIs at maxilloturbinate (p <0.05) and nasal turbinate and lateral wall (p <0.01), compared to controls
Level III: no increases in ULLIs</td></tr>
<tr><td>7.9</td><td>NR</td></tr>
</table>
[a]Based on data from 3 to 5 rats per exposure group and 10 to 12 control rats.
<table>
<tr><th>FA (mg/m^3)</th><th>Cell proliferation measured by BrdU after 3 days[a]</th></tr>
<tr><td>1.2</td><td>Levels II and III: no increases in ULLIs</td></tr>
<tr><td>3.9</td><td>Levels II and III: no increases in ULLIs</td></tr>
<tr><td>7.9</td><td>NR</td></tr>
</table>
[a]Based on data from 3 to 5 rats per exposure group and 10 to 12 control rats.

This study also evaluated the combined effects of FA, acetaldehyde, and acrolein on nasal epithelium. Data presented here are for formaldehyde-only exposed rats</td></tr>
</table>

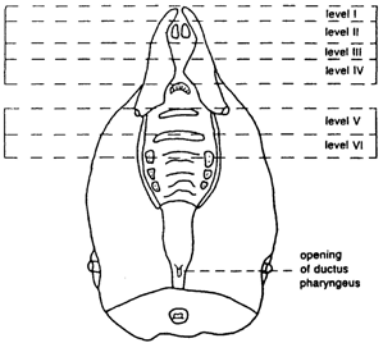
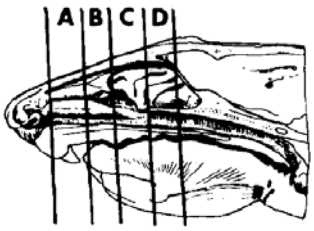
<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td>


Figure 1 from Cassee et al. (1996b) depicting cross levels of the rat nose evaluated for cell proliferation.
</td><td></td></tr>
<tr><td>
Chang et al. (1983); [additional data from related Swenberg et al. (1983b) report]
Fischer 344 rats; males; 4–5/exposure group, 9/control group.
Exposure: Rats were exposed to FA in head-only chambers 6 hrs/d for 1, 3, 5, or 10 d.
Test article: Paraformaldehyde.
Actual concentrations were 0 and 18.5 (±0.1) mg/m^3.[1] Target concentrations were 0, 0.62, 2.46, 3.69, 7.38, 14.76, or 18.45 mg/m^3 in Swenberg et al. (1983b) report.
Cell proliferation studies carried out after FA exposure with [^{3}H]thymidine labeling (i.p. injection 2 or 18 hrs postexposure) and scoring of cells (n=9,000) lining the respiratory epithelium from the nasal and maxillary turbinates and lateral wall.


Levels A (with minimal mucociliary clearance) and B (with extensive mucociliary clearance) reported in Swenberg et al. (1983b)
</td><td>
<table>
<tr><th>Group (18.5 mg/m^3)</th><th>Labeling index (%) in Level B</th></tr>
<tr><td>Control</td><td>0.43±0.05 (9)[a]</td></tr>
<tr><td>1 day</td><td>5.51±0.35 (4)[b]</td></tr>
<tr><td>5 days</td><td>10.05±0.27 (5)[b, c]</td></tr>
</table>
[a]Number in parentheses represents number of animals studies; [b]Significantly different from control, p<0.05; [c]Significantly different from 1-d exposed rats, p<0.05.

% labeled respiratory epithelial cells in Level B (thymidine at 2 h postexposure)
<table>
<tr><th></th><th colspan="5">Formaldehyde Concentration (mg/m^3)</th></tr>
<tr><th>Duration</th><th>0</th><th>0.62</th><th>2.46</th><th>7.38</th><th>18.45</th></tr>
<tr><td>3 days</td><td>0.22 (0.03)</td><td>0.38 (0.05)</td><td>0.33 (0.06)</td><td>5.4 (0.82)</td><td>2.83 (0.81)</td></tr>
</table>
% labeled respiratory epithelial cells (thymidine at 18 h postexposure)
<table>
<tr><th></th><th>3 d (Level B)</th><th>10 d (Level B)</th><th>3 d (Level A)</th></tr>
<tr><td>Control</td><td>0.54 (0.03)</td><td>0.26 (0.02)</td><td>3.0 (1.56)</td></tr>
<tr><td>3.69 mg/m^3 × 12 hr/d</td><td>1.73 (0.63)</td><td>0.49 (0.19)</td><td>16.99 (1.5)</td></tr>
<tr><td>7.38 mg/m^3 × 6 hr/d</td><td>3.07 (1.09)</td><td>0.53 (0.2)</td><td>15.46 (10.01)</td></tr>
<tr><td>14.76 mg/m^3 × 3 hr/d</td><td>9.0 (0.88)</td><td>1.73 (0.65)</td><td>16.49 (2.07)</td></tr>
</table>
Mean (SEM); Group sizes and statistical comparisons not reported in Swenberg et al. (1983b)

Note: Pulse labeling with thymidine 18 hrs compared to 2 hrs postexposure resulted in ≈2-fold and ≈3-fold increase in labeling in control rats and at 7.38 mg/m^3, respectively (Swenberg et al., 1983b).
</td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td>Kuper et al. (2011)
Fischer 344 rats; male; 8/group.
Exposure: Mice were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for 4 wks.
Test article: Formalin (10.21% FA).
Actual concentrations were 0, 0.63 (±0.06), 1.23 (±0.14), 2.48 (±0.18), 7.53 (±0.42), 12.3 (±0.48), and 18.4 (±0.06) mg/m^3.[1]

Cell proliferation studies conducted with surgical implantation of BrdU-containing pumps (3 d prior to sacrifice) and determining labeling index of 2 sections of NALT and 1 section of an upper-respiratory tract-draining lymph node (i.e., posterior and superficial cervical lymph nodes). Cell proliferation data reported as BrdU-positive cells per length (i.e., mm) of epithelium.</td><td>Lymph nodes: No FA-related effects on the number of BrdU-positive cells reported in the follicle and paracortex compartments and medulla

BrdU counts in section 1 of NALT
<table><tr><th>FA (mg/m^3)</th><th>Interfollicular area</th><th>Interfollicular epithelium</th><th>Follicular area</th><th>Follicular epithelium</th></tr><tr><td>0</td><td>61.9±18.8[a]</td><td>6.5±3.2</td><td>73.0±39.1</td><td>12.6±17.5</td></tr><tr><td>0.63</td><td>57.3±17.4</td><td>4.9±2.2</td><td>53.5±19.4</td><td>4.9±3.8</td></tr><tr><td>1.23</td><td>55.7±17.7</td><td>5.9±3.4</td><td>52.2±27.9</td><td>6.4±6.5</td></tr><tr><td>2.48</td><td>53.5±12.9</td><td>4.3±2.7</td><td>49.8±22.1</td><td>4.7±3.2</td></tr><tr><td>7.53</td><td>51.1±14.9</td><td>3.3±2.4</td><td>47.6±13.9</td><td>5.8±5.3</td></tr><tr><td>12.3</td><td>55.5±15.3</td><td>5.5±3.5</td><td>51.2±16.2</td><td>5.7±2.9</td></tr><tr><td>18.4</td><td>54.4±11.6</td><td>28.2±11.1[b]</td><td>41.4±14.2</td><td>23.6±13.6[c]</td></tr></table>[a]Mean number of BrdU-positive cells±SD; [b]p <0.001; [c]p <0.05.

BrdU counts in section 2 of NALT
<table><tr><th>FA (mg/m^3)</th><th>Interfollicular area</th><th>Interfollicular epithelium</th><th>Follicular area</th><th>Follicular epithelium</th></tr><tr><td>0</td><td>48.3±17.7[a]</td><td>6.3±2.2</td><td>62.3±24.1</td><td>6.8±1.5</td></tr><tr><td>0.63</td><td>51.0±16.3</td><td>4.4±2.7</td><td>58.0±30.5</td><td>5.8±5.6</td></tr><tr><td>1.23</td><td>53.9±12.2</td><td>4.1±2.9</td><td>47.0±15.3</td><td>6.9±3.8</td></tr><tr><td>2.48</td><td>53.4±14.2</td><td>5.1±2.4</td><td>52.2±15.1</td><td>5.6±4.0</td></tr><tr><td>7.53</td><td>48.2±12.3</td><td>3.5±2.3</td><td>47.2±15.0</td><td>5.9±2.8</td></tr><tr><td>12.3</td><td>56.0±16.3</td><td>6.4±2.3</td><td>56.8±17.4</td><td>6.2±4.7</td></tr><tr><td>18.4</td><td>49.9±9.1</td><td>24.5±12.6[b]</td><td>40.1±11.8</td><td>22.9±10.5[b]</td></tr></table>[a]Mean number of BrdU-positive cells±SD; [b]p<0.001.</td></tr>
<tr><td>Monticello et al. (1991) Fischer 344 rats; males; 4–6/group.
Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for 1, 4, or 9 d or 6 wks.
Test article: Paraformaldehyde.
Actual concentrations were 0, 0.85 (±0.01), 2.48 (±0.02), 7.63 (±0.12), 12.2 (±0.11), and 18.2 (±0.28) mg/m^3.[1]
Cell proliferation studies carried out after FA exposure with [^{3}H]thymidine labeling (ip injection 18 hrs postexposure) and profiling nasal epithelial cells in serial sections of Levels II and III of the nose. Level II included the lateral meatus with the lateral aspect of the nasoturbinate, lateral wall, and lateral aspect of maxilloturbinate (Site 1); midseptum (Site 2); and medial aspect of maxilloturbinate (Site 3). Level</td><td>Mean until length labeling indices[a]
<table><tr><th></th><th></th><th></th><th colspan="4">Exposure time</th></tr><tr><th>mg/m^3</th><th>Level</th><th>Site</th><th>1 d</th><th>4 d</th><th>9 d</th><th>6 wks</th></tr><tr><td>0</td><td>II</td><td>1</td><td>2.16[b]</td><td>1.46</td><td>1.44</td><td>0.91</td></tr><tr><td></td><td></td><td>2</td><td>1.08</td><td>1.03</td><td>1.09</td><td>0.41</td></tr><tr><td></td><td></td><td>3</td><td>2.49</td><td>1.36</td><td>1.38</td><td>1.02</td></tr><tr><td></td><td>III</td><td>1</td><td>1.83</td><td>1.10</td><td>1.36[c]</td><td>0.98</td></tr><tr><td></td><td></td><td>2</td><td>3.02</td><td>2.81</td><td>1.68[c]</td><td>2.18</td></tr><tr><td>0.85</td><td>II</td><td>1</td><td>1.31[c, e]</td><td>1.37</td><td>1.20</td><td>0.88[c]</td></tr><tr><td></td><td></td><td>2</td><td>1.01[c]</td><td>0.97</td><td>0.80</td><td>0.24[c]</td></tr><tr><td></td><td></td><td>3</td><td>1.75[c]</td><td>1.54</td><td>0.80</td><td>1.21[c]</td></tr><tr><td></td><td>III</td><td>1</td><td>1.72[c]</td><td>1.27</td><td>1.40</td><td>0.91[c]</td></tr><tr><td></td><td></td><td>2</td><td>1.74[c]</td><td>3.09</td><td>1.06</td><td>1.54[c]</td></tr><tr><td>2.48</td><td>II</td><td>1</td><td>2.36[c]</td><td>1.72</td><td>1.73</td><td>1.36</td></tr><tr><td></td><td></td><td>2</td><td>1.69[c]</td><td>0.67</td><td>0.97</td><td>0.68</td></tr><tr><td></td><td></td><td>3</td><td>2.81[c]</td><td>1.09</td><td>1.48</td><td>1.11</td></tr><tr><td></td><td>III</td><td>1</td><td>2.46[c]</td><td>1.09[c]</td><td>1.74</td><td>0.86</td></tr><tr><td></td><td></td><td>2</td><td>2.39[c]</td><td>1.43[c]</td><td>1.43</td><td>2.57</td></tr></table></td></tr>
</table>

Reference and study design	Results

III included the lateral wall (Site 1) and midventral septum (Site 2).

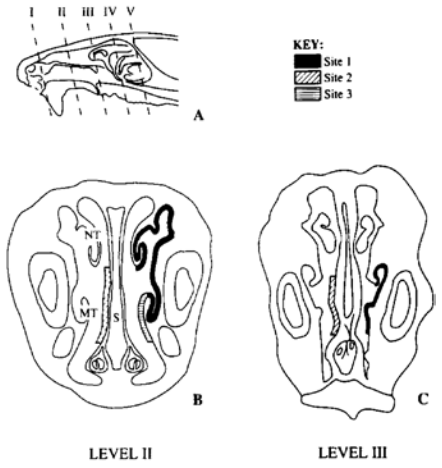


Figure 1 from Monticello et al. (1991). (A) Lateral view of the rat nose with Levels I–V of the nasal passage. (B) Level II and (C) Level III represent sites for cell proliferation studies.

7.63	II	1	16.86 [c, f, g]	30.51 [f, g]	23.51 [f, g]	14.41 [f, g]
		2	3.85 [c]	10.00 [f]	10.85 [f]	2.10
		3	18.15 [c, f]	25.03 [f]	22.54 [f]	16.32 [f]
	III	1	7.53 [f]	8.77 [c, f]	7.35 [f]	2.08
		2	4.20	9.22 [c, f]	9.50 [f]	2.58
12.2	II	1	11.17 [c, f]	20.91 [f]	28.59 [f]	23.87 [c, f]
		2	17.90 [c, f]	26.12 [f, g]	19.62 [f]	21.44 [c, f, g]
		3	5.87 [c]	20.26 [f]	20.95 [f]	26.07 [c, f]
	III	1	14.48 [f]	20.01 [c, f]	30.59 [f]	24.21 [f]
		2	24.44 [f]	18.70 [c, f]	28.60 [f]	13.98 [f]
18.2	II	1	12.68 [f]	25.78 [f]	24.57 [c, f]	28.74 [c, f]
		2	16.72 [f]	29.10 [f]	29.09 [c, f]	25.95 [c, f]
		3	5.31	19.39 [f]	28.71 [c, f]	25.10 [c, f]
	III	1	16.35 [d, f]	30.80 [c, f]	40.36 [f]	34.78 [c, f]
		2	19.26 [d, f]	34.43 [c, f]	32.53 [f]	27.47 [c, f]

[a]Unit length labeling index defined as the number of labeled cell profiles/mm basement membrane; [b]n=6, unless otherwise indicated; [c]n=5; [d]n=4; [e]Unless noted, not statistically different from control; [f] p <0.05 compared to control; [g] p <0.05 compared to level III.

Reuzel et al. (1990)

Wistar rats; male; 5/group.

Exposure: Rats were exposed in dynamic whole-body chambers 22 hrs/d for 3 d to FA.

Test article: Paraformaldehyde.

Actual concentrations were 0, 0.37 (±0.01), 1.4 (±0.0), and 3.8 (±0.1) mg/m^3 FA.[1]

Cell proliferation studies carried out after FA exposure with [^{3}H]thymidine labeling (ip injection 2 hrs postexposure) and scoring of the cells lining the nasal (n=1,000) and maxillary (n=1,000) turbinates, lateral wall (n=1,000), and the septum (n=2,000).

See diagram from Cassee et al. (1996b) (above) for cross levels of the rat nose evaluated for cell proliferation.

Data extracted using GrabIt software (mean from level 2, Figure 3, HCHO only):

mg/m^3	*Maxilloturb.*	*Nasal Turb.*	*Lateral wall*	*septum*
0	0.351855128	0.291340043	1.19765084	0.172349
0.369	0.287744031	0.842204054	1.04583032	0.221581
1.23	0.221580704	0.337503123	0.54215496	0.221581
3.69	4.456151692*	5.273729396*	5.8261316*	4.627466*

Note: data were also presented for Level 3 (same regions). While slight increases became noticeable at 3.69 mg/m^3, none reached statistical significance.

This study also evaluated the combined effects of FA and ozone mixtures on nasal epithelium. Ozone co-exposure resulted in an increase in proliferation compared to formaldehyde exposure alone. Data are only presented herein for formaldehyde-only exposures.

Roemer et al. (1993)

Sprague Dawley rats; male; 3 or 5/exposure group, 6 or 10/control group.

Proportion of BrdU-labeled cells (%) after exposure

		Formaldehyde (mg/m^3)			
Cell origin and exposure frequency	*Number of rats per group[a]*	*0*	*2.5*	*7.4*	*24.6*

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td>Exposure: Rats were exposed to FA in dynamic head-only chambers 6 hrs/d for 1 or 3 d.
Test article: Paraformaldehyde.
Actual concentrations were within 10% of nominal concentrations of 0, 2.5, 7.4, or 24.6 mg/m^3.[1]
Cell proliferation studies carried out after FA exposure with BrdU labeling (i.p. injection 16–22 hrs postexposure) and flow cytometry analysis of 10,000 cells per measurement.</td><td>
<table>
<tr><td>Nose</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>1 exposure</td><td>5</td><td>1.3 (0.1)[b]</td><td>2.4 (0.6)[c]</td><td>3.7 (0.5)[c]</td><td>2.7 (0.8)[c]</td></tr>
<tr><td>3 exposures</td><td>5</td><td>NR</td><td>1.4 (0.3)</td><td>2.5 (0.2)[c]</td><td>2.3 (0.2)[c]</td></tr>
<tr><td>Trachea</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>1 exposure</td><td>5</td><td>1.2 (0.1)</td><td>3.1 (0.6)[c]</td><td>2.1 (0.8)</td><td>2.8 (0.4)[c]</td></tr>
<tr><td>3 exposures</td><td>5</td><td>NR</td><td>0.3 (0.1)[c]</td><td>0.6 (0.1)[c]</td><td>2.5 (0.2)[c]</td></tr>
<tr><td>Lung</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>1 exposure</td><td>3</td><td>1.8 (0.3)</td><td>2.6 (0.6)</td><td>3.3 (0.4)</td><td>3.1 (0.7)</td></tr>
<tr><td>3 exposures</td><td>3</td><td>NR</td><td>2.2 (0.0)</td><td>2.4 (0.7)</td><td>5.1 (1.5)</td></tr>
</table>
[a]Twice the number of rats in control groups; [b]Standard error in parentheses;
[c]Statistically significant at $p \leq 0.05$, compared with controls.
</td></tr>
<tr><td>Wilmer et al. (1987)
Wistar rats; male; 10/group.
Exposure: Rats were exposed to FA (chamber type not reported) either continuously for 8 hrs/d, 5 d/wk for 4 wks or intermittently 8 hrs/d (successive periods of 0.5 hr of exposure and 0.5 hr of nonexposure), 5 d/wk for 3 d and 4 wks.
Test article: Paraformaldehyde.
Actual concentrations were not determined. Target concentrations were 0, 6.2, or 12.3 mg/m^3 for continuous exposures and 0, 12.3, or 24.6 mg/m^3 for intermittent exposures.[1]
Cell proliferation studies carried out after 3 d or 4 wks of FA exposure with [^{3}H]thymidine labeling (ip injection 18 hrs postexposure) and scoring of the cells (n=5,000) lining the nasal and maxillary turbinates, the septum, and the lateral wall.</td><td>
Percentage of [^{3}H]thymidine labeled cells in nasal epithelium
<table>
<tr><th></th><th></th><th colspan="2">% labeled cells</th></tr>
<tr><th>Exposure</th><th>Exposure x time</th><th>After 3 d of exposure (n=3)</th><th>After 4 wks of exposure (n=3)</th></tr>
<tr><td>0 mg/m^3</td><td>0 mg/m^3 hr/d</td><td>0.86 (0.14)[a]</td><td>0.68 (0.12)</td></tr>
<tr><td>6.2 mg/m^3 (continuous)</td><td>49.6 mg/m^3 hr/d</td><td>2.82 (0.47)[b]</td><td>1.33 (0.75)</td></tr>
<tr><td>12.3 mg/m^3 (continuous)</td><td>98.4 mg/m^3 hr/d</td><td>8.87 (1.51)[b]</td><td>8.85[c]</td></tr>
<tr><td>12.3 mg/m^3 (intermittent)</td><td>49.2 mg/m^3 hr/d</td><td>9.80 (1.54)[d]</td><td>3.41 (1.25)[e]</td></tr>
<tr><td>24.6 mg/m^3 (intermittent)</td><td>98.4 mg/m^3 hr/d</td><td>19.77 (2.39)[d]</td><td>13.87 (0.64)[d]</td></tr>
</table>
[a]SDs shown in parentheses; [b]$p<0.01$, compared to controls; [c]Data from one rat; [d]$p<0.001$, compared to controls; [e]$p<0.05$, compared to controls.
</td></tr>
<tr><td colspan="2">Medium Confidence</td></tr>
<tr><td>Cassee and Feron (1994) Wistar rats; male; 20/group.
Exposure: Rats were exposed in dynamic nose-only chambers for 3 d (6 consecutive 12-hr periods of 8 hrs of exposure to FA followed by 4 hrs of nonexposure). Rats sacrificed immediately (i.e., within 30 min) after last exposure.
Test article: Paraformaldehyde.
Actual concentrations were 0 and 4.4 (SE ±0.1) mg/m^3 FA alone.[1]</td><td>
<table>
<tr><th></th><th colspan="2">Controls</th><th colspan="2">FA alone[a]</th></tr>
<tr><th>Site</th><th>II[b]</th><th>III[b]</th><th>II</th><th>III</th></tr>
<tr><td>Nasoturbinates</td><td>+[c]</td><td>+</td><td>+++</td><td>+++</td></tr>
<tr><td>Maxilloturbinates</td><td>+</td><td>+</td><td>+++</td><td>+++</td></tr>
<tr><td>Septum</td><td>+</td><td>+</td><td>+++</td><td>+++</td></tr>
<tr><td>Lateral wall</td><td>+</td><td>+</td><td>+++</td><td>+++</td></tr>
</table>
[a]Only nonnecrotic areas at cross level II showed severe PCNA expression; [b]Standard cross level II and III through the nose; [c]PCNA-expression scores: +, some nuclei stained; ++, a moderate number of nuclei stained; +++, many nuclei stained.
</td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td>Cell proliferation studies carried out using deparaffinized standard cross sections of the nose and semiquantitative proliferating cell nuclear antigen (PCNA) immunostaining.

See diagram from Cassee et al. (1996b) (above) for cross sections of a rat nose examined for PCNA staining by Cassee and Feron (1994).</td><td>In animals exposed to FA alone, no increased PCNA staining observed in olfactory epithelium.

This study also evaluated the combined effects of FA and ozone mixtures on nasal epithelium. Ozone co-exposure resulted in an increase in proliferation compared to formaldehyde exposure alone. Data are only presented herein for formaldehyde-only exposures.</td></tr>
<tr><td>Speit et al. (2011b)
Fischer 344 rats; males; 6/group.
Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for 4 wks.
Test article: Formalin (methanol concentration NR).
Actual concentrations were 0, 0.63 (±0.6), 1.23 (±0.14), 2.48 (±0.18), 7.53 (±0.42), 12.3 (±0.48), 18.4 (±0.06) mg/m^3.[1]

Cell proliferation studies conducted with surgical implantation of BrdU-containing pumps (3 days prior to sacrifice) and determining labeling index of 3 levels of the nasal cavity: I (nasal septum, lateral meatus [wall], maxilloturbinate, nasoturbinate), II (nasal septum, lateral meatus [wall]), and IV (nasopharynx). Cell proliferation data reported as BrdU-labeled nuclei per mm of basal lamina (i.e., ULLI).</td><td>ULLI for level III not assessed due to author's expectation that this level was not a sensitive target tissue.

ULLI for nasal level I
<table><tr><th>mg/m^3</th><th>Nasal septum</th><th>Lateral meatus</th><th>Maxillo-turbinate</th><th>Naso-turbinate</th></tr><tr><td>0</td><td>6.64±1.30[a]</td><td>8.44±3.37</td><td>10.21±5.90</td><td>14.15±2.93</td></tr><tr><td>0.63</td><td>8.02±2.57</td><td>10.80±1.58[b]</td><td>9.49±3.07</td><td>17.13±6.97</td></tr><tr><td>1.23</td><td>6.04±2.20</td><td>9.56±3.68</td><td>10.43±5.52</td><td>22.60±5.86[c]</td></tr><tr><td>2.48</td><td>6.14±3.15</td><td>11.56±4.73</td><td>9.08±2.65</td><td>14.29±5.59</td></tr><tr><td>7.53</td><td>4.80±3.14</td><td>14.85±2.40[c]</td><td>12.95±3.94</td><td>20.48±8.12[b]</td></tr><tr><td>12.3</td><td>3.83±2.13</td><td>52.53±16.30[c]</td><td>52.42±16.88[c]</td><td>74.63±28.90[c]</td></tr><tr><td>18.4</td><td>70.86±14.30[c]</td><td>74.21±16.37[c]</td><td>81.96±2.90[c]</td><td>67.50±12.76[c]</td></tr></table>[a]Group mean value±SD; [b]p<0.05; [c]p<0.01.

<table><tr><th colspan="3">*ULLI for nasal level II*</th><th>*ULLI for nasal level IV*</th></tr><tr><th>mg/m^3</th><th>Nasal septum</th><th>Lateral meatus</th><th>Naso-pharynx</th></tr><tr><td>0</td><td>14.59±6.37[a]</td><td>9.33±4.22</td><td>17.81±2.18[a]</td></tr><tr><td>0.63</td><td>19.93±7.66</td><td>7.58±2.32</td><td>21.23±5.19</td></tr><tr><td>1.23</td><td>22.36±7.04[b]</td><td>8.04±2.92</td><td>21.56±3.17</td></tr><tr><td>2.48</td><td>21.79±5.28[b]</td><td>9.47±3.31</td><td>21.33±3.55[b]</td></tr><tr><td>7.53</td><td>19.07±6.43</td><td>9.28±3.54</td><td>20.93±4.13</td></tr><tr><td>12.3</td><td>26.66±11.31</td><td>37.13±5.22[c]</td><td>29.23±4.25[c]</td></tr><tr><td>18.4</td><td>62.36±12.30[c]</td><td>55.21±10.99[c]</td><td>73.29±15.87[c]</td></tr></table>[a]Group mean value±SD; [b]p <0.05; [c]p <0.01.

Relative change (% control) in ULLI in metaplastic/ degenerative (M) and nonmetaplastic (O) epithelia
<table><tr><th></th><th colspan="2">Nasal septum</th><th colspan="2">Lateral meatus</th><th colspan="2">Maxillo-turbinate</th><th colspan="2">Naso-turbinate</th></tr><tr><th>mg/m^3</th><th>M</th><th>O</th><th>M</th><th>O</th><th>M</th><th>O</th><th>M</th><th>O</th></tr><tr><td>Level I</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr><tr><td>12.3</td><td>58</td><td>61</td><td>622[a,b]</td><td>1195[a]</td><td>513[a,c]</td><td>262[a]</td><td>527[a,c]</td><td>139</td></tr></table></td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td></td><td>
<table>
<tr><td>18.4</td><td>1066[a]</td><td>1386[a]</td><td>879[a,c]</td><td>1399[a]</td><td>802[a]</td><td>735[a]</td><td>477[a,b]</td><td>280[d]</td></tr>
<tr><td colspan="9">Level II</td></tr>
<tr><td>12.3</td><td>183</td><td>161</td><td>398[a,c]</td><td>110</td><td>NA</td><td>NA</td><td>NA</td><td>NA</td></tr>
<tr><td>18.4</td><td>428[a,c]</td><td>1188[a]</td><td>592[a,c]</td><td>195[a]</td><td>NA</td><td>NA</td><td>NA</td><td>NA</td></tr>
</table>
[a]p <0.01, compared to corresponding untreated control; [b]p <0.05, comparison between metaplastic and nonmetaplastic tissues; [c]p<0.01, comparison between metaplastic and nonmetaplastic tissues; [d]p <0.05, compared to corresponding untreated control.
</td></tr>
<tr><td>Woutersen et al. (1987)
Wistar rats; male and female; 10/sex/group.
Exposure: Rats were exposed to FA in dynamic whole-body chambers for 6 hrs/d, 5 d/wk for 3 d.
Test article: Paraformaldehyde.
Actual concentrations were 0, 1.2 (±0.00), 11.9 (±0.15), and 24.4 (±0.09) mg/m^3.[1]
Cell proliferation studies carried out after 3 d of FA exposure with [^{3}H]thymidine labeling of dissected nasoturbinates (18 hrs postexposure) and scoring of the cells (n=1,000) of the respiratory epithelium.</td><td>
Percentage of [^{3}H]thymidine labeled cells in nasal epithelium (males, n=2/group)
<table>
<tr><th></th><th colspan="2">% labeled cells</th></tr>
<tr><th>mg/m^3</th><th>Visibly unaffected epithelium</th><th>Metaplastic epithelium</th></tr>
<tr><td>0</td><td>1.6 (1.2–2.0)[a]</td><td>NR</td></tr>
<tr><td>1.2</td><td>1.2 (0.8–1.5)</td><td>NR</td></tr>
<tr><td>11.9</td><td>2.6 (1.4–3.8)</td><td>31.4 (29.5–33.2)</td></tr>
<tr><td>24.4</td><td>2.8[b]</td><td>37.6 (32.6–42.5)</td></tr>
</table>
[a]Range in parentheses; [b]Value based on one rat since most respiratory epithelium was metaplastic.
</td></tr>
<tr><td colspan="2">Mice</td></tr>
<tr><td colspan="2">High Confidence</td></tr>
<tr><td>Chang et al. (1983) [additional data from related Swenberg et al. (1983b) report]
B6C3F1 mice; males; 4–5/exposure group, 10/control group.
Exposure: Mice were exposed to FA in head-only chambers 6 hr/d for either 1, 3, 5 or 10 d.
Test article: Paraformaldehyde.
Actual concentrations were 0 and 18.5 (±0.1) mg/m^3. Target concentrations were 0, 0.62, 2.46, 3.69, 7.38, 14.76 or 18.45 mg/m^3 in Swenberg et al. (1983b) report.
Cell proliferation studies carried out after FA exposure with [^{3}H]thymidine labeling (ip injection 2 or 18 hrs postexposure) and scoring of cells (n=4,000) lining the respiratory</td><td>
<table>
<tr><th>Group (18.5 mg/m^3)</th><th>Labeling index (%) in Level B</th></tr>
<tr><td>Control</td><td>0.27±0.04 (10)[a]</td></tr>
<tr><td>1 day</td><td>2.14±0.56 (5)[b]</td></tr>
<tr><td>5 days</td><td>3.42±0.84 (4)[b]</td></tr>
</table>
[a]Number in parentheses represents number of animals studies.
[b]Significantly different from control, p <0.05.

% labeled respiratory epithelial cells in Level B (thymidine at 2 hr postexposure)
<table>
<tr><th></th><th colspan="5">Formaldehyde Concentration (mg/m^3)</th></tr>
<tr><th></th><th>0</th><th>0.62</th><th>2.46</th><th>7.38</th><th>18.45</th></tr>
<tr><td>3 days</td><td>0.12 (0.02)</td><td>0.09 (0.04)</td><td>0.08 (0.04)</td><td>0.15 (0.06)</td><td>0.97 (0.04)</td></tr>
</table>
% labeled respiratory epithelial cells in Level A (thymidine at 18 hr postexposure)
<table>
<tr><td>Control</td><td>1.24 (0.57)</td></tr>
<tr><td>3.69 mg/m^3 × 12 hr/d for 10 d</td><td>10.14 (3.20)</td></tr>
<tr><td>7.38 mg/m^3 × 6 hr/d for 10 d</td><td>4.72 (1.61)</td></tr>
</table>
</td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td>epithelium from the nasal and maxillary turbinates and lateral wall.

See diagram from Swenberg et al. (1983b) for rats (above) for locations of Levels A (with minimal mucociliary clearance) and B (with extensive mucociliary clearance)</td><td>
<table>
<tr><td>14.76 mg/m^3 × 3 hr/d for 10 d</td><td>1.76 (0.49)</td></tr>
</table>
Mean (SEM); Group sizes and statistical comparisons not reported in Swenberg et al. (1983b)
</td></tr>
<tr><td>Kuper et al. (2011)
B6C3F1 mice; females; 6/group.
Exposure: Mice were exposed to FA in dynamic whole-body chambers 6 hr/d, 5 d/wk for 4 wk.
Test article: Formalin (10.21% FA).
Actual concentrations were 0, 0.63 (±0.06), 1.23 (±0.14), 2.48 (±0.18), 7.53 (±0.42), 12.3 (±0.48), and 18.4 (±0.06) mg/m^3.[1]

Cell proliferation studies conducted with surgical implantation of BrdU-containing pumps (3 d prior to sacrifice) and determining labeling index of 2 sections of NALT and 1 section of a upper-respiratory tract-draining lymph node (i.e., posterior and superficial cervical lymph nodes). Cell proliferation data reported as BrdU-positive cells per length (i.e., mm) of epithelium.</td><td>NALT: No FA-related effects on the number of BrdU-positive cells reported in the follicular and interfollicular compartments and epithelium

Lymph nodes: No FA-related effects on the number of BrdU-positive cells reported in the follicle and paracortex compartments and medulla</td></tr>
<tr><td colspan="2">Monkeys</td></tr>
<tr><td colspan="2">Medium Confidence</td></tr>
<tr><td>Monticello et al. (1989)
Rhesus monkeys; male; 3/group.
Exposure: Monkeys were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for 1 or 6 wks.
Test article: Paraformaldehyde.
Actual concentrations were not determined. Target concentration was 7.4 mg/m^3. Controls were sham exposed to biologically filtered air for 6 wks.[1]
Cell proliferation studies carried out after FA exposure with [^{3}H]thymidine labeling (iv injection 18 hrs postexposure) and scoring of respiratory epithelial cells. For nasal passages</td><td>
<table>
<tr><th>Exposure</th><th>Observations between nasal passage epithelia</th></tr>
<tr><td>Controls (6 wk)</td><td>Highest LIs in transitional epithelium compared to respiratory and olfactory epithelia</td></tr>
<tr><td>7.4 mg/m^3 (1 wk)</td><td>Transitional and respiratory epithelia elevated compared to controls (p ≤0.05)</td></tr>
<tr><td>7.4 mg/m^3 (6 wk)</td><td>Transitional epithelium LIs slightly elevated over controls and had decreased from 1-wk group; olfactory epithelium LIs had mild increase over controls (p ≤0.05); respiratory epithelium LIs elevated compared to controls (p ≤0.05)</td></tr>
</table>
<table>
<tr><th>Exposure</th><th>Observations between levels of nasal passages</th></tr>
<tr><td>Controls (6 wk)</td><td>LIs for Levels B–E significantly increased over controls (p ≤0.05), anterio-posterior gradient (i.e., greatest to lowest) in cell proliferation rates</td></tr>
</table>
</td></tr>
</table>

Reference and study design	Results

(transitional, respiratory, and olfactory epithelia), larynx, trachea, and carina, LIs defined as the number of labeled cells per mm of basal lamina.

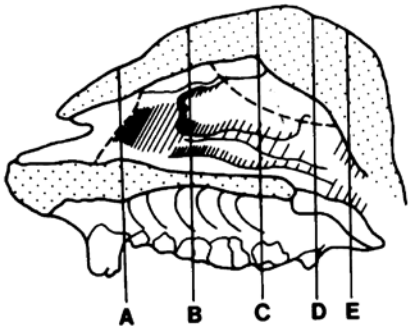


Figure 4 from (Monticello et al., 1989) depicting the nasal passage levels selected for cell proliferation studies. A, nasal atrium; B, anterior aspect of the middle and ventral turbinates; C, mid-region of the maxillary sinuses; D, posterior nasal cavity; and E, nasopharynx.

7.4 *mg/m³* (1 wk)	LIs for Levels B–E significantly increased over controls (p≤0.05)
7.4 *mg/m³* (6 wk)	Levels C–E significantly elevated over 1-wk group (p ≤0.05)

Group	*Observations within levels of nasal passages*
Level A	NR
Level B	LIs for 1- and 6-wk groups elevated over controls (*p* ≤0.05) for septum, inferior meatus, inferior turbinate, lateral wall, and middle turbinate
Level C	LIs for 1- and 6-wk groups elevated over controls (*p* ≤0.05) for septum, inferior meatus, inferior turbinate, lateral wall, and middle turbinate; no increase in LIs for 1- and 6-wk groups over controls for maxillary sinuses
Level D	LIs for 1-wk group elevated over controls (*p* ≤0.05) for septum, inferior meatus, inferior turbinate, and lateral wall; LIs for 6-wk group elevated over controls (*p* ≤0.05) for inferior meatus and inferior turbinate
Level E	LIs for 1-wk group elevated over controls (*p* ≤0.05) for floor and lateral and dorsal walls; LIs for 6-wk group elevated over controls (*p*≤0.05) for septum, floor, and lateral and dorsal walls

Group	*Observations for nonnasal tissues*
Larynx	LIs for 1- and 6-wk groups elevated over controls; LIs increased with duration of exposure
Trachea	Significant elevation in LIs for 1-wk (*p* ≤0.05) but not 6-week group over controls; LIs increased with duration of exposure
Carina	Significant elevation in LIs for 1-wk (*p* ≤0.05) but not 6-wk group over controls; LIs increased with duration of exposure

Interanimal variation in LIs for trachea and carina

Exposure	*Animal #*	*Trachea LI*	*Carina LI*
Controls (6 wk)	1	0.29	0.42
	2	0.46	0.37
	3	0.91	0.50
	ave	0.55±0.19[a]	0.43±0.04[a]
7.4 *mg/m³* (1 wk)	4	1.34	1.09
	5	0.90	1.95
	6	1.19	0.99
	ave	1.14±0.13[a]	1.34±0.31[a]
7.4 *mg/m³* (6 wk)	7	8.00	3.86
	8	2.30	6.49
	9	0.88	0.45
	ave	3.73±2.18[a]	3.60±1.75[a]

[a]Represents Mean±SEM.

Reference and study design	Results
	Exposure | *LI in respiratory bronchioles*[a] Controls (6 wk) | 0.01±0.001 7.4 *mg/m*^{3} (1 wk) | 0.01±0.003 7.4 *mg/m*^{3} (6 wk) | 0.01±0.001 [a]LIs expressed as percent labeled cells per total cell count from ≥500 respiratory bronchiolar nucleated epithelial cells per animal.

Changes in the LRT

Although the URT and the LRT are physically and functionally connected, this analysis delineates findings across these two tissue compartments. This was done due to the distribution of the overwhelming majority of inhaled formaldehyde to the URT (noting that some data suggest that oronasal breathing in humans, as compared to nose-only breathing in rodents, might result in slight differences in the distribution of inhaled formaldehyde, including a possible increase in the portion reaching proximal regions of the LRT such as the trachea; see Appendix A.2). Thus, evidence related to studies of BAL (bronchoalveolar lavage) fluid and airway function, both of which may involve some contribution from URT-related changes but are largely driven by effects on the lung, are described in this section. The specific studies and summary findings supporting the synthesis below are described in Table A-78. In general, compared to effects on the URT, the methodological approaches for evaluating LRT changes are more commonly applied to studies of exposed humans, so this section considers a wider range of evidence. A greater level of concern exists for the erroneous attribution of changes in the LRT (and other, non-URT, compartments in subsequent sections) to inhaled formaldehyde when studies used methanol-containing formalin; thus, findings from some studies using exposure paradigms similar to those described in the previous section are interpreted with comparably less confidence.

As previously mentioned, formaldehyde-induced stimulation of TRPA1 receptors on trigeminal nerve endings distributed within the epithelial cell layer in the URT appears to cause a localized release of neuropeptides, including substance P, which can cause local inflammatory changes. Consistent with this, ex vivo models of LRT tissues and *low confidence* studies of in vivo exposure suggest that indirect activation of sensory nerve endings in the LRT, presumably of the vagus nerve, occurs after formaldehyde inhalation exposure. In the URT, this activation is expected to occur via direct interaction of formaldehyde with receptors. However, while these direct interactions might occurn in upper portions of the LRT during certain, very rare human exposure scenarios (e.g., in the trachea at high exposure levels), they would be unexpected in the lungs or during typical exposure scenarios; thus, this is not considered a plausible initial effect of typical exposure. Notwithstanding this assumption, the available evidence indicates that formaldehyde exposure likely causes downstream sequelae in the lung that could be attributed to sensory nerve activation in the LRT, predominantly related to substance P-related pathways (see below).

However, the mechanistic event(s) critical to understanding this potential relationship remain unknown: namely, how sensory nerve endings in the LRT would be stimulated without distribution of inhaled formaldehyde to the LRT. The most likely explanations involve a secondary response to TRP channel-activating stimuli increased via other mechanisms, such as increased LRT oxidative stress and/or inflammatory mediators released from activated immune cells or damaged epithelial cells in the LRT. It could also be explained by a central trigeminal-to-vagal neural reflex response to irritation of the URT (i.e., a "nasobronchial" reflex[20]); however, the existence of this reflex in humans is debated and a clear scientific consensus does not exist (Giavina-Bianchi et al., 2016; Sahin-Yilmaz and Naclerio, 2011; Togias, 2004, 1999). No studies specifically designed to assess any of these potential linkages after formaldehyde exposure were identified.

Studies in several species provide moderate evidence that formaldehyde exposure results in increased LRT neuropeptides, including substance P (see "Changes in the URT" Section above), as well as a rapid activation of the primary receptor for substance P, the neurokinin receptor (NK_1R), typically at formaldehyde concentrations ≥2.5 mg/m^3. Further, the activation of this pathway has been experimentally linked to both formaldehyde-induced leakage of the LRT microvasculature (which has been observed in rodents at ≥1.23 mg/m^3) as well as airway hyperresponsiveness (which has been observed in animals and humans at <0.5 mg/m^3). In addition to facilitating the recruitment of inflammatory cells, NK_1R activation can promote immune cell survival and activation through the release of cytokines and chemokines (Tuluc et al., 2009). The substance P-NK_1R pathway has been implicated in mast cell degranulation, which can lead to bronchoconstriction (Bienenstock and Mcdermott, 2005); however, while inhibiting mast cell activation prevented microvascular leakage in a *low confidence* rat study after acute exposure to high levels of formaldehyde (Kimura et al., 2010), an acute *medium* or *high confidence* study of a cohort of guinea pigs failed to observe any changes in mast cells (Swiecichowski et al., 1993; Leikauf, 1992). Importantly, an understanding of potential changes to substance P and NK1R-dependent effects (e.g., due to desensitization) with long-term formaldehyde exposure remains unclear. While a transient depletion of neuropeptides from sensory nerve terminals after acute exposure seems plausible (Leikauf, 1992). Importantly, an understanding of potential changes to substance P and NK1R-dependent effects (e.g., due to desensitization) with long-term formaldehyde exposure remains unclear. While a transient depletion of neuropeptides from sensory nerve terminals after acute exposure seems plausible (Kimura et al., 2010), substance P is still elevated, at least in the blood, after subchronic exposure (Fujimaki et al., 2004b). Overall, the activation characteristics of this pathway in the LRT across various formaldehyde exposure scenarios have not been established.

[20] Note: neural reflexes involving afferent and efferent activity of the vagus nerve (e.g., across different LRT regions), some of which may involve C fibers and TRP channels, are better established (Mazzone and Undem, 2016).

Microvascular leakage can lead to inflammatory structural changes observable by histology, which are supported by *moderate* evidence in formaldehyde-exposed rodents, particularly those sensitized with the allergen, ovalbumin (OVA). The available studies indicate changes including airway edema (swelling) or thickening of airway walls, with general support for inflammatory changes in airway bronchi, but not necessarily alveoli. In addition, the pattern of structural changes varied across studies, with a study in guinea pigs observing airway swelling without signs of inflammation at low formaldehyde (<0.5 mg/m^3) levels (Riedel et al., 1996), while studies in rats and mice generally observed mild inflammatory-related structural changes at higher levels (i.e., ≥3.0 mg/m^3) that only became pronounced with allergen sensitization. It is important to note that animal models vary in their ability to mimic some features of human airways. Airway responses in guinea pigs often differ from those in rats and mice, and while no animal model fully recapitulates human airway function, in many ways the sensitivity of guinea pig airways may be more relevant than other small mammals (e.g., similar structure of the lung to humans; responsiveness to stimuli that induce sensitivity in humans) (Shin et al., 2009; Ricciardolo et al., 2008). Alongside airway inflammation and structural changes, including edema, which could narrow or obstruct airways, an increased permeability to bronchoconstrictors such as histamine would be expected to influence airway function, possibly linking these changes to observations of hyperresponsiveness or decreased pulmonary function.

A *moderate* association between formaldehyde exposure and increases in LRT eosinophils was identified, including amplification of the response of these cells in rodents previously exposed to allergens (see Table A-79). Taken together with similar findings in the URT, a general increase in airway eosinophils as a result of formaldehyde exposure is supported by robust evidence. As in the URT, this finding has been reported in the LRT following exposure for several weeks at effective concentrations above 0.5 mg/m^3. The only study of longer-term exposure available (Fujimaki et al., 2004b) indicated that formaldehyde exposure at 2.46 mg/m^3, but not ≈0.5 mg/m^3, for 3 months caused increased eosinophils in mice sensitized to OVA, but not in unsensitized mice. While the data are not conclusive, it appears that eosinophil recruitment does not occur immediately after acute exposure, as this increase was not observed in the available studies of acute exposure (see Table A-79). Although it has not been mechanistically demonstrated based on increased eosinophils and other immune cells after acute tachykinin release (Barnes, 1998, 1992), repeated release of neuropeptides could plausibly lead to sustained airway inflammation and, depending on the phenotype of the recruited cells, this could result in airway hyperresponsiveness. In both the URT and LRT, recruitment of eosinophils might also be related to changes in markers of oxidative stress observed across formaldehyde exposure paradigms. However, whereas oxidative stress in the URT may be related to damage to the local epithelial cells, most studies indicate that formaldehyde exposure does not result in overt damage to the LRT airway epithelium (*slight* evidence, at relatively high formaldehyde levels: >5 mg/m^3), making this potential linkage less

plausible. It is considered more likely that increases in oxidative stress are the result of changes in inflammatory factors and immune cells in the LRT, rather than LRT epithelial damage.

The evidence for LRT immunological changes other than those seen in eosinophils is mixed and generally only suggestive of potential effects. As shown in Figure A-34, *slight* evidence exists to suggest that formaldehyde exposure amplifies recruitment of innate immune cells such as neutrophils and monocytes to the LRT; notably, this finding has only been observed when animals exposed to >2 mg/m^3 were previously sensitized to an allergen. Importantly, few studies examined lymphocyte subsets, and no studies reported on the response of lymphocytes in animals sensitized to allergens or at exposure levels below 5 mg/m^3, highlighting important gaps in the literature. Two studies suggest that CD8$^+$, but not CD4$^+$, T cells may be increased with formaldehyde exposure above 7 mg/m^3 (Jung et al., 2007; Sandikci et al., 2007b). The only study meeting the inclusion criteria that evaluated lymphocyte changes in both immature and adult animals only observed changes in animals exposed as adults (Sandikci et al., 2007b), which could suggest that a functionally mature immune system is necessary for these alterations (the immune system is not considered to be fully mature in rodents until around six weeks of age (Burns-Naas et al., 2008)). While these findings should be interpreted with substantial caution, there may be a role for CD8$^+$ T cells in promoting the recruitment and survival of airway eosinophils, as well as a requirement of these cells for the development of airway hyperresponsiveness (e.g., to allergen or infection) (Schwarze et al., 1999; Hamelmann et al., 1997). CD8$^+$ T cells make up a heterogeneous population of lymphocytes which migrate by recruitment to sites of inflammation, proliferate in response to antigen stimulation, and help to mediate long-term cellular immunity against foreign pathogens, particularly viruses. The conventional role for IFNγ-producing CD8$^+$ T cells is to inhibit eosinophil function; however, some emerging evidence suggests that certain CD8$^+$ T cell subpopulations may induce eosinophil recruitment (Huber and Lohoff, 2015). No data are available to evaluate the potential for effects of formaldehyde exposure on different subpopulations of LRT CD8$^+$ T cells.

Studies of markers of immune cell activation in the LRT after formaldehyde exposure generally provide mixed results, making it difficult to draw inferences (see Table A-79). Most cytokine-related changes reported in the LRT occur at high formaldehyde levels (>5 mg/m^3) after short-term exposure and include *slight* evidence to support an increase in eosinophil chemotactic factors, and a decrease in markers and counts of natural killer (NK) cells. NK cells respond rapidly to infection and appear to have a role in regulating chronic inflammation and infection of the airways (FJ, 2009). Thus, this change, were it to be experimentally verified, could be associated with the *moderate* evidence of an increased propensity for LRT infections, similar to the *slight* evidence of altered URT immune responses (see previous section); however, definitive studies relevant to long-term exposure have not been identified and additional data are necessary to interpret these alterations in respiratory immune responses as consistent with immune suppression. A number of consistent studies in exposed rodents do suggest an increase in T helper type 2 (Th2)-related cytokines, most notably IL-4, with short term exposure at ≥0.5 mg/m^3 and

particularly in animals sensitized to an allergen. The *slight* evidence supporting increased IL-5, a Th2 cytokine that can be both synthesized by and act upon airway mast cells and eosinophils and which is believed to be integral to the development of airway eosinophilia and airway hyperresponsiveness (Greenfeder et al., 2001; Schwarze et al., 1999), is considered to be inconclusive (i.e., two *low confidence* studies testing exposure levels >5 mg/m^3). Along with IL-5 and IL-13, IL-4 is recognized for its established role in chronic respiratory disorders (Maes et al., 2012), and this change may be relevant to other LRT-specific changes. IL-4, which can stimulate T cell receptors on CD4$^+$ and CD8$^+$ T cells (Serre et al., 2010), can influence the activation and development of antigen-specific CD8$^+$ T cell immunity by shifting the phenotype of these cells from IFN-γ production to IL-4 production (Erb and Le Gros, 1996).

The cytokine changes could be related to the *moderate* evidence for increased LRT infections and the *slight* evidence suggesting reduced NK cell numbers (see Tables A-79 and A-73), as Th2 cytokines have been shown to reduce pulmonary bacterial immunity (Beisswenger et al., 2006) and NK cells have a role in regulating chronic inflammation and infection of the airways (FJ, 2009). A key limitation of the data is that the few formaldehyde-specific studies have not demonstrated consistent increases in CD4$^+$ Th2 cells in the airways of exposed individuals. Similarly, interactions between airway innate and adaptive immune responses, and between CD4$^+$ and CD8$^+$ T cells, topics of current interest (Gasteiger and Rudensky, 2014; Koya et al., 2007), have not been well studied following formaldehyde exposure. Experiments focused on these types of endpoints would help to integrate the currently available data.

The consistent evidence of amplified airway responses to immunogenic stimuli (e.g., to allergens such as OVA) following formaldehyde exposure is of particular interest. As described above, multiple LRT parameters are affected or exacerbated by the combination of formaldehyde exposure and sensitization to allergenic materials. At concentrations ranging from 0.31–3 mg/m^3 over durations of several days to several weeks, formaldehyde exposure in combination with allergen sensitization exacerbates immune-related changes, such as: recruitment of eosinophils and possible increases in IL-4; airway structural changes, including edema; and airway functional changes, including exaggerated responses to muscarinic receptor agonists. These observations may be relevant to the associations between human formaldehyde exposure at much lower concentrations (<0.05 mg/m^3) and conditions that may reflect an enhanced response to allergens (e.g., rhinoconjunctivitis; asthma).

The formaldehyde exposure-induced effects associated with allergen sensitization varied depending on the specific mechanistic effect and the experimental animal model. This variability may reflect a lack of consistency in the methods used for sensitization and challenge, or other experimental design differences across studies. Alternatively, these differences might reflect variability in susceptibility to these types of effects across different populations or groups of individuals (e.g., animals of different species, strains, sex, or age). This variable sensitivity of subsets of the population to formaldehyde-induced effects would be consistent with observations

of substantial interindividual human variability for several potential health effects. Further, these data suggest that vulnerability to some formaldehyde-induced health effects might be influenced by the exposure history of the individuals, including exposure to known allergens. The mechanism for this amplified response to allergens (and, possibly, nonallergenic antigens) due to formaldehyde exposure, including what airway component(s) formaldehyde may interact with to initiate this particular alteration, remains unknown. Possible explanations include formaldehyde acting as an antigen (capable of directly eliciting an antibody response) or as a hapten (capable of eliciting an antibody response when bound to a larger molecule such as a protein), or formaldehyde-induced chronic inflammation acting as an adjuvant (enhancing immune responses to antigens); however, these speculations have not been examined by directed testing following inhalation exposure. While changes in airway responsiveness could be dependent on stimulation of sensory nerve endings, observations in isolated tracheae by Swiecichowski et al. (1993) and Leikauf (1992) suggest that the amplified response to stimuli is at least partly mediated by interactions with local immuno-modulatory factors. As airway hyperreactivity and other indicators of immunologic sensitization are known to be related to markers (e.g., antibodies) in the blood, some evidence related to these responses are discussed in the subsequent section. Overall, the essential airway immunologic target(s) of inhaled formaldehyde has not yet been identified and verified, thereby presenting a key uncertainty.

Table A-78. Summary of changes in the lower respiratory tract (LRT) as a result of formaldehyde exposure

Endpoint		Study-specific findings from "high or medium" or "low" confidence experiments	Summary of evidence (exposure duration)	Conclusion
Structural Modification of the Lower Airways				
Microvascular Leakage	High or Medium	*Human*: None *Animal*: Increased in rats (Ito et al., 1996): acute at ≥6.15 mg/m^3; note: inhibited at 18.45 mg/m^3 by NK1 receptor antagonist (note: substance P binds NK_1R), but not histamine or bradykinin antagonists	Demonstrated increased leakage from acute exposure ≥6.15 mg/m^3 in 1 study, which might be mediated by substance P	**Moderate** ↑ only examined in acute studies
	Low	*Human*: None *Animal*: Transiently increased in rats (Kimura et al., 2010): acute at ≥1.23 mg/m^3 (duration-independent); Note: leakage blocked by inhibiting mast cells, but not blocking cyclooxygenases; potential additional mechanistic understanding by injection of formalin into the trachea causing leakage that appeared to be dependent on substance P release after stimulation of C-fiber afferents (Lundberg and Saria, 1983)	One study suggests acute exposure as low as 1.23 mg/m^3 induces microvascular leakage, although continued exposure appeared to (at least in the near-term) result in less leakage	
Airway Edema and/or Other Inflammatory Structural Change	High or Medium	*Human*: None *Animal*: Increased edema in lung bronchi, but not alveoli, without signs of inflammation in lower airways in guinea pigs (Riedel et al., 1996): 5 d at 0.31 mg/m^3, not 0.16 mg/m^3	Bronchial edema in 1 short-term study at 0.31 mg/m^3	**Moderate** ↑ may require higher exposure levels and/or allergen sensitization for pronounced changes
	Low	*Human*: None *Animal*: Airway structural changes consistent with inflammation (e.g., wall thickening; cell infiltration) in mice (Jung et al., 2007); (Wu et al., 2013; Liu et al., 2011) and in mice and rats sensitized with OVA (Wu et al., 2013; Liu et al., 2011; Qiao et al., 2009), but not in nonsensitized rats (Qiao et al., 2009): all 2–3 wk at ≥3 mg/m^3 [Note: most studies indicated assessment of bronchial airways]	Airway structural changes with allergen sensitization in 2 species (and, to a lesser extent, without sensitization) with short-term exposure at ≥3 mg/m^3	
Airway/Airway Epithelial Cell Damage	High or Medium	*Human*: None *Animal*: N/C (histology for mouse epithelial cell damage) (Fujimaki et al., 2004b): 12 wk at up to 2.46 mg/m^3 N/C in histology in guinea pigs (Swiecichowski et al., 1993; Leikauf, 1992): acute at 4.18 mg/m^3	N/C in a single mouse subchronic study with i.p. sensitization and up to 2.46 mg/m^3 exposure, nor in a guinea pig study at 4.18 mg/m^3	**Slight** at higher formaldehyde levels

Endpoint	Study-specific findings from "high or medium" or "low" confidence experiments		Summary of evidence (exposure duration)	Conclusion
	Low	*Human*: None	A single short-term study in mice and another in rats, and indirect evidence from several studies in rats, suggests damage at higher formaldehyde levels (e.g., around 4 mg/m^3); however, another similar study did not observe effects at 12.3 mg/m^3	
		Animal: Increased in mice (Jung et al., 2007): 2 wk at ≥6.15 mg/m^3 and in rats (Aydin et al., 2014): 4 wk at ≥6.15 mg/m^3; indirect evidence of damage in rats ((Kimura et al., 2010) *and* (Dallas et al., 1987) *and* (Sandikci et al., 2009)): 20 hr after acute at 6.15 mg/m^3 *and* 1 wk at ≥0. 62 mg/m^3 (effect magnitude decreased with longer exposures) *and* 6 wk at 7.38 mg/m^3 (in adults, not young), and in mice (Abreu et al., 2016): 6–8 hr after acute at 3.7 mg/m^3, but N/C in rats in another study (Dinsdale et al., 1993): 4 d at 12.3 mg/m^3		

Table A-78. Summary of changes in the lower respiratory tract (LRT) as a result of formaldehyde exposure (continued)

Endpoint		Study-specific findings from "high or medium" or "low" confidence experiments	Summary of evidence (exposure duration)	Conclusion
LRT Sensory Nerve Activation	High or Medium	*Human*: None	No evidence to evaluate	**Slight** levels required for potential activation unknown (note: may involve TRPA1 binding)
		Animal: None		
	Low	*Human*: None	A single acute rat study and indirect evidence from potentially related exposures suggest that lower airway sensory nerve afferents may be activated, but the inhaled formaldehyde levels required for such potential activation have not been experimentally demonstrated	
		Animal: With acute exposure, dose-dependent increase in nerve currents and Cl^- release in intact rat trachea (Luo et al., 2013), with supporting evidence of substance P and NK Receptor involvement. Indirectly, increased substance P and CGRP were observed in mouse lung tissue, both were amplified with OVA, and both were dependent on TRP activation (Wu et al., 2013): short term at 3 mg/m^3. Note: the potential involvement of tracheobronchial reflexes in the pulmonary effects of cigarette smoke constituents, such as nicotine and formalin, may add indirect support		
Immune and Inflammation-Related Changes				
[[See Table A-79 for Cellular and Cytokine Response in BAL and LRT tissues]]				
Oxidative Stress	High or Medium	*Human*: Increased exhaled nitric oxide, a noninvasive marker of lower airway inflammation and oxidative stress, in healthy or asthmatic children (Flamant-Hulin et al., 2010; Franklin et al., 2000): unknown duration (likely months to years: classrooms or homes) at 0.04–0.06 mg/m^3, but not in elderly nursing home patients at lower levels (Bentayeb et al., 2015): unknown duration (likely months to years) at 0.005–0.01 mg/m^3	Increased biomarkers (indirect evidence) of oxidative stress in children at ≥0.04 mg/m^3, but not in elderly individuals at ≤0.01 mg/m^3 with prolonged (months–years) exposure	**Moderate** ↑ in children at ≈0.04 mg/m^3
		Animal: None		
	Low	*Human*: None		

Table A-78. Summary of changes in the lower respiratory tract (LRT) as a result of formaldehyde exposure (continued)

Endpoint	Study-specific findings from "high or medium" or "low" confidence experiments		Summary of evidence (exposure duration)	Conclusion
		Animal: in mice: NO and NOS activity increased with 3 d at 3 mg/m^3 (Yan et al., 2005), GSH levels decreased with 3 wk at ≥0.5 mg/m^3 (Ye et al., 2013), and increased ROS and/or lipid peroxidation markers with 3 wk at ≥1 mg/m^3 (Ye et al., 2013) or 2 wk at ≥6.15 mg/m^3 (Jung et al., 2007), but decreased with acute exposure in 1 study (Matsuoka et al., 2010): 24 hr at 0.12 mg/m^3 in rats: at ≥12.3 mg/m^3 increased total oxidant levels and decreased total antioxidant level (Aydin et al., 2014): 4 wk, increased lipid peroxidation markers and protein oxidation markers (Sul et al., 2007): 2 wk, and decreased gamma-glutamyl transpeptidase- indirect evidence (Dinsdale et al., 1993): 4 d	Multiple studies in two species suggest elevated oxidative stress at ≥1 mg/m^3 with short-term exposure	
Sustained Inflammation	High or Medium	*Human*: Increased exhaled nitric oxide, a noninvasive marker of lower airway inflammation and oxidative stress, in healthy or asthmatic children (Flamant-Hulin et al., 2010; Franklin et al., 2000): unknown duration (likely months to years: classrooms or homes) at 0.04–0.06 mg/m^3	Immune cell counts are continually elevated in a subchronic mouse study with allergen stimulation at 2.46 mg/m^3; increased biomarkers (indirect evidence) of lower airway inflammation are observed in children with prolonged exposure.	**Moderate** may require allergen sensitization in some cases
		Animal: Eosinophils and monocyte counts remain elevated with continued exposure for subchronic duration with allergen (OVA) sensitization (Fujimaki et al., 2004b): 12 wks at 2.46 mg/m^3		
	Low	*Human*: None	BAL cell counts and histologic evidence suggest that inflammation persists for several weeks with short-term exposure, and these effects are amplified by allergen	
		Animal: Immune cell counts were increased with short term exposure in several studies at ≥0.5 mg/m^3 (see Table A-79); histological evidence of inflammation without epithelial damage was noted in short-term studies, typically at higher concentrations, which were amplified by allergen (e.g., ≥3 mg/m3, Wu et al., 2013; Kimura et al., 2010)		
Immune Function	High or Medium	*Human*: Increased LRT infections in infants (Roda et al., 2011): 32–41% increase in incidence per 0.0124 mg/m^3 increase in formaldehyde (LOD: 0.008 mg/m^3); ≈1-yr exposure at 0.020 mg/m^3 (median)	Indirect evidence in a single study of infants exposed to a median of 0.020 mg/m^3 that observed an association	**Moderate** supports an increased

Table A-78. Summary of changes in the lower respiratory tract (LRT) as a result of formaldehyde exposure (continued)

Endpoint		Study-specific findings from "high or medium" or "low" confidence experiments	Summary of evidence (exposure duration)	Conclusion
(inferred from LRT infections)		*Animal*: Decreased antibacterial activity in mice (Jakab, 1992): acute at 1.23 mg/m^3, noting that this finding appeared to be particularly sensitive to the pattern of formaldehyde exposure	between exposure and increased infections. One acute mouse study also provided indirect support for an increased likelihood of respiratory infections.	propensity for LRT infections, particularly during development
	Low	*Human*: Increased emergency room visits for episodes including LRT infections (Rumchev et al., 2002): children aged 6–36 mos with mean levels of 0.028–0.030 (maximum 0.12–0.22) mg/m^3	Direct and indirect evidence of impaired LRT immune function in children and in a short-term rat study, respectively	
		Animal: Decreased expression of immune-related genes in rat lung (Sul et al., 2007), specifically HSP701a (may be involved in antigen presentation), complement 4 binding protein (may bind necrotic or apoptotic cells for cleanup), and Fc portion of IgGiii (may be involved in leukocyte activation): 2 wk at ≥6.15 mg/m^3		
Changes in pulmonary function with challenge (e.g., with broncho-constrictors and/or allergens) (Note: unprovoked responses are not included)	High or Medium	*Human*: None	Acute and short-term studies in two animal species demonstrate that formaldehyde increases responsiveness to allergens and bronchoconstrictors, particularly with prior sensitization, at levels as low as 0.31 mg/m^3	**Robust** ↑ Hyperresponsive airways[a] (↑ effects with allergen)
		Animal: [allergen challenge]: With ovalbumin [OVA] sensitization, increased airway obstruction in guinea pigs (Riedel et al., 1996): short-term at 0.31 mg/m^3 and increased reactivity in mice (Larsen et al., 2013): acute at ≈5–7 mg/m^3 in humid or dry environments; [acetylcholine challenge]: Increased airway resistance and reactivity in guinea pigs (Swiecichowski et al., 1993; Leikauf, 1992): acute at 1.23 mg/m^3		
	Low	*Human*: [histamine challenge]: Hyperreactive airways with prolonged exposure (Górski and Krakowiak, 1991): ≥1 year at ≤0.5 mg/m^3, but N/C after acute exposure (Krakowiak et al., 1998): 2 hr at 0.5 mg/m^3; [allergen challenge]: hypersensitivity with acute exposure when exposure was restricted to mouth breathing in allergic asthmatics with a large allergen (mite) (Casset et al., 2006): ≤1 hr at 0.1 mg/m^3, but N/C after acute oronasal (normal) exposure in allergic asthmatics using a different allergen (pollen), including a test of methacholine (MCh) responsiveness 8 hr after allergen exposure (Ezratty et al., 2007): 1 hr at 0.5 mg/m^3	***Suggestive*** evidence of increases with prolonged exposure, and possibly acute mouth-breathing exposure when challenged with specific allergens, but not acute exposure alone, to ≤0.5 mg/m^3 in human adults; also, increased at ≥3 mg/m^3 in short-term or acute studies across three species, particularly with prior sensitization	

Table A-78. Summary of changes in the lower respiratory tract (LRT) as a result of formaldehyde exposure (continued)

Endpoint	Study-specific findings from "high or medium" or "low" confidence experiments		Summary of evidence (exposure duration)	Conclusion
		Animal: [MCh challenge]: Hyperresponsive airways (increased reactivity and sensitivity) noted with FA alone in mice and rats (Wu et al., 2013; Liu et al., 2011; Qiao et al., 2009): short-term at ≥3 mg/m^3, and in monkeys (Biagini et al., 1989): acute at 3.1 mg/m^3; in mice and rats, this response was amplified with OVA sensitization; Note: TRP antagonists reduced the hyperresponsiveness in mice (Wu et al., 2013)		

[a]As the challenge stimuli used in the formaldehyde studies included allergens as well as nonimmunological stimuli, and because most experiments did not attempt to delineate the specifics of the functional changes, "airway hyperresponsiveness" or "hyperresponsive airways" encompasses any of a range of possible airway features: hyperreactivity (exaggerated response), hypersensitivity (lower dose to elicit response), altered ventilatory parameters (e.g., maximal response; resistance), recovery (longevity of response), or others.

Table A-79. Summary of changes in LRT cell counts and immune factors as a result of formaldehyde exposure

Endpoint(s)			No changes observed (*high or medium confidence* experiments are bolded)		Significant[a] increases or decreases (*high or medium confidence* experiments are bolded)		Summary conclusion Clarifying notes and exposure duration
			Duration (species)	Concentration(s) [allergen stimulus] (study)	*Duration (species)*	Concentration(s) [allergen stimulus] (study)	
White blood cells (WBCs)	Total WBCs (or Total Inflammatory Cells)		***Acute (g pigs)*** *Acute (humans)* *Acute (mice)* *Acute (mice)*	**0.13–0.31 mg/m³ [–OVA] (Riedel et al., 1996)** 0.5 mg/m³ [+ pollen] (Ezratty et al., 2007) 0.5–6.2 mg/m³ [–OVA] (Larsen et al., 2013) 0.25–3.7 mg/m³ [–OVA] (Abreu et al., 2016)	***Subchronic (mice)*** *Short term (mice)* *Short term (mice)* *Short term (mice)* *Short term (rats)*	**↑ 2.5 mg/m³ [+ OVA] (Fujimaki et al., 2004b)** ↑ 12.3 mg/m³ [–OVA] (Kim et al., 2013a); total BAL cells ↑ 12.3 mg/m³ [–OVA] (Jung et al., 2007) ↑ 3 mg/m³ [± OVA] (Wu et al., 2013) ↑ 0.5–3.1 mg/m³ [+ OVA] (Qiao et al., 2009)	**Moderate ↑** short-term ≥0.5 mg/m³; amplifies allergen effect
	Granulocytes	Neutrophils	***Subchronic (mice)*** ***Acute (g pigs)*** *Short term (mice)* *Acute (humans)*	**0.1–2.5 mg/m³ [± OVA] (Fujimaki et al., 2004b)** **4.2 mg/m³ [–OVA] (Swiecichowski, 1993, 43200)** 6.2–12.3 mg/m³ [–OVA] (Jung et al., 2007) 0.5 mg/m³ [+ pollen] (Ezratty et al., 2007)	*Short term (mice)* *Acute (rats)*	↑ 3 mg/m³ [+ OVA] (Wu et al., 2013) ↑ 6.2 mg/m³ [–OVA] (Kimura et al., 2010)	**Slight ↑** amplifies allergen response at >3 mg/m³ (short-term)

Endpoint(s)			No changes observed (*high or medium confidence* experiments are bolded)		Significant[a] increases or decreases (*high or medium confidence* experiments are bolded)		Summary conclusion Clarifying notes and exposure duration
			Duration (species)	**Concentration(s) [allergen stimulus] (study)**	***Duration (species)***	**Concentration(s) [allergen stimulus] (study)**	
		Eosinophils	*Acute (humans)* *Acute (humans)* *Acute (rats)*	(trend ↑) 0.1 mg/m^3[+ Der^b f] (Casset et al., 2007) 0.5 mg/m^3 [+ pollen] (Ezratty et al., 2007) 6.2 mg/m^3 [−OVA] (Kimura et al., 2010)	***Subchronic (mice)*** *Short term (mice)* *Short term (mice)* *Short term (mice)* *Short term (mice)* *Short term (rats)*	**↑ 2.5 mg/m^3 [+ OVA] (Fujimaki et al., 2004b)** ↑ 12.3 mg/m^3 [−OVA] (Jung et al., 2007) ↑ 0.5−3 mg/m^3 [± OVA] (Liu et al., 2011) ↑ 3 mg/m^3 [± OVA] (Wu et al., 2013) ↑ infer[1] >12.3 mg/m^3 [+ Der f] (Sadakane et al., 2002) ↑ 0.5−3.1 mg/m^3 [+ OVA] (Qiao et al., 2009)	**Moderate ↑** short-term ≥0.5 mg/m^3; amplifies allergen effect
	Lymphocytes	**All**	***Subchronic (mice)*** *Short term (mice)* *Short term (mice)* *Acute (humans)*	**0.1−2.5 mg/m^3 [± OVA] (Fujimaki et al., 2004b)** 6.2−12.3 mg/m^3 [−OVA] (Kim et al., 2013a) 12.3 mg/m^3 [−OVA] (Jung et al., 2007) 0.5 mg/m^3 [+ pollen] (Ezratty et al., 2007)	*Short term (mice)*	↑ 3 [−OVA] mg/m^3 (Wu et al., 2013)	**Indeterminate** suggests total number unchanged
		B Cells	***Acute (g pigs)*** *Short term (mice)* *Short term (mice*	**4.2 mg/m^3 [−OVA] (Swiecichowski et al., 1993)** 6.2−12.3 mg/m^3 [−OVA] (Kim et al., 2013a) (trend ↓) 6.2−12.3 mg/m^3 [−OVA] (Jung et al., 2007)			**Indeterminate** allergen stimulus unstudied

Endpoint(s)			No changes observed (*high or medium confidence* experiments are bolded)		Significant[a] increases or decreases (*high or medium confidence* experiments are bolded)		Summary conclusion Clarifying notes and exposure duration
			Duration (species)	**Concentration(s) [allergen stimulus] (study)**	***Duration (species)***	**Concentration(s) [allergen stimulus] (study)**	
		T Cells ($CD4^+$)	*Short term (mice)* *Short term (mice)*	6.2–12.3 mg/m^3 [–OVA] (Kim et al., 2013a) (trend ↑) 6.2–12.3 mg/m^3 [–OVA] (Jung et al., 2007)	*Short term (rats)*	↑ (adults) 7.4 mg/m^3 [–OVA] (Sandikci et al., 2007b)	**Indeterminate** allergen stimulus unstudied
		T Cells ($CD8^+$)	*Short term (mice)*	6.2–12.3 mg/m^3 [–OVA] (Kim et al., 2013a)	*Short term (rats)* *Short term* (mice)	↑ (adults) 7.4 mg/m^3 [–OVA] (Sandikci et al., 2007b) ↑ (slight) 12.3 mg/m^3 [–OVA] (Jung et al., 2007)	**Slight↑** short-term >7 mg/m^3, allergen stimulus unstudied
		NK Cells			*Short term (mice)*	↓ 12.3 mg/m^3 [–OVA] (Kim et al., 2013a)	**Indeterminate**
	Monocytes		***Acute (g pigs)*** *Acute (humans)* *Acute (rats)*	**4.2 mg/m^3 [–OVA] (Swiecichowski et al., 1993)** 0.5 mg/m^3 [+ pollen] (Ezratty et al., 2007) 6.2 mg/m^3 [–OVA] (Kimura et al., 2010)	***Subchronic (mice)***	**↑ 2.5 mg/m^3 [+ OVA] (Fujimaki et al., 2004b)**	**Slight↑** long-term ≥2.5 mg/m^3 amplifies allergen effect
Mast Cells			***Acute (g pigs)***	**4.2 mg/m^3 [–OVA] (Swiecichowski et al., 1993)**			**Indeterminate**
Secreted factors and immune	**Primarily Th1-related**	**TNF-α and GM-CSF**	***Subchronic (mice)*** *Acute (humans)* *Acute (mice)*	**0.1–2.5 mg/m^3 [± OVA] (Fujimaki et al., 2004b)** 0.5 mg/m^3 [+ pollen] (Ezratty et al., 2007) 0.25–3.7 mg/m^3 [–OVA] (Abreu et al., 2016)			**Indeterminate** suggests unchanged or highly variable

Endpoint(s)			No changes observed (*high or medium confidence* experiments are bolded)		Significant[a] increases or decreases (*high or medium confidence* experiments are bolded)		Summary conclusion Clarifying notes and exposure duration
			Duration (species)	**Concentration(s) [allergen stimulus] (study)**	***Duration (species)***	**Concentration(s) [allergen stimulus] (study)**	
		IFN-γ	*Short term (mice)* *Short term (mice)* *Acute (humans)*	0.5–3 mg/m³ [± OVA] (Liu et al., 2011) 3 mg/m³ [± OVA] (Wu et al., 2013) 0.5 mg/m³ [+ pollen] (Ezratty et al., 2007)	*Short term (mice)* *Short term (rats)*	↓ 6.2–12.3 mg/m³ [–OVA] (Kim et al., 2013a) ↑ 3.1 mg/m³ [–OVA] (Qiao et al., 2009)	
		IL-1 (IL-1β in animals)	*Acute (humans)* *Acute (mice)*	0.5 mg/m³ [+ pollen] (Ezratty et al., 2007) 0.25–3.7 mg/m³ [–OVA] (Abreu et al., 2016)	***Subchronic (mice)*** *Short term (mice)* *Short term (mice)*	**↓ 2.5 mg/m³ [+ OVA]** (Fujimaki et al., 2004b) ↑ 3 mg/m³ [–OVA] (Wu et al., 2013) ↑ 6.2–12.3 mg/m³ [–OVA] (Jung et al., 2007)	
	Primarily Th2-related	**IL-4**	*Short term (mice)* *Acute (humans)*	infer[a] >12.3 mg/m³ [± Der f] (Sadakane et al., 2002) 0.5 mg/m³ [+ pollen] (Ezratty et al., 2007)	*Short term (mice)* *Short term (mice)* *Short term (mice)* *Short term (mice)* *Short term (rats)*	↑ 1–3 mg/m³ [–OVA] (Lu et al., 2005) ↑ 6.2–12.3 mg/m³ [–OVA] (Jung et al., 2007) ↑ 0.5–3 [+ OVA] or 3 [–OVA] mg/m³ (Liu et al., 2011) ↑ 3 mg/m³ [± OVA] (Wu et al., 2013) ↑ 0.5–3.1 mg/m³ [+ OVA]; ↓ 3.1 mg/m³ [–OVA] (Qiao et al., 2009)	**Slight↑** IL-4 at ≥0.5 mg/m³ and IL-5 at ≥6.15 mg/m³, short-term and likely amplifying allergen effects
		IL-5	*Acute (humans)*	0.5 mg/m³ [+ pollen] (Ezratty et al., 2007)	*Short term (mice)* *Short term (mice)*	↑ 6.2–12.3 mg/m³ [–OVA] (Jung et al., 2007) ↑ infer[a] >12.3 mg/m³ [+ Der f] (Sadakane et al., 2002)	
		IL-10	*Acute (humans)*	0.5 mg/m³ [+ pollen] (Ezratty et al., 2007)			**Indeterminate**

Endpoint(s)			No changes observed (*high or medium confidence* experiments are bolded)		Significant[a] increases or decreases (*high or medium confidence* experiments are bolded)		Summary conclusion Clarifying notes and exposure duration
			Duration (species)	**Concentration(s) [allergen stimulus] (study)**	***Duration (species)***	**Concentration(s) [allergen stimulus] (study)**	
		IL-6	***Subchronic (mice)*** *Acute (mice)*	**0.1–2.5 mg/m³ [± OVA] (Fujimaki et al., 2004b)** 0.25–3.7 mg/m³ [−OVA] (Abreu et al., 2016)	*Short term (mice)*	↑ 0.5–3 [+ OVA] or 3 [−OVA] mg/m³ (Liu et al., 2011)	
		IL-13	*Short term (mice)*	6.2–12.3 mg/m³ [−OVA] (Jung et al., 2007)			
	NK cell factors	**IL-2R**			*Short term (mice)*	↓ 6.2–12.3 mg/m³ (Kim et al., 2013a)	**Indeterminate**
		Perforin					
	Eosinophil attractant and adhesion factors	**RANTES**			*Short term (mice)*	↑ infer[a] >12.3 mg/m³ [± Der f] (Sadakane et al., 2002)	**Slight↑** chemoattractants relevant to eosinophil recruitment with short-term exposure
		ICAM and CCR3			*Short term (mice)*	↑ (indirect[b]) 12.3 mg/m³ [−OVA] (Jung et al., 2007)	
		Eotaxin	***Subchronic (mice)*** *Acute (humans)*	**0.1–2.5 mg/m³ [± OVA] (Fujimaki et al., 2004b)**[3] 0.5 mg/m³ [+ pollen] (Ezratty et al., 2007)	*Short term (mice)*	↑ (indirect[b]) 12.3 mg/m³ [−OVA] (Jung et al., 2007)	
		ECP	*Acute (humans)*	0.5 mg/m³ [+ pollen] (Ezratty et al., 2007)	*Acute (humans)*	↑ 0.1 mg/m³ [+ Der f] (Casset et al., 2007)	
		MIP-1α	***Subchronic (mice)***	**0.1–2.5 mg/m³ [± OVA] (Fujimaki et al., 2004b)**[3]			
	Other	**IL-8**	*Acute (humans)*	0.5 mg/m³ [+ pollen] (Ezratty et al., 2007)	*Acute (in vitro)*	↑ 1.23 mg/m³ (Rager et al., 2011)	**Indeterminate**

Endpoint(s)			No changes observed (*high or medium confidence* experiments are bolded)		Significant[a] increases or decreases (*high or medium confidence* experiments are bolded)		Summary conclusion Clarifying notes and exposure duration
			Duration (species)	**Concentration(s) [allergen stimulus] (study)**	***Duration (species)***	**Concentration(s) [allergen stimulus] (study)**	
		MCP-1	***Subchronic (mice)*** *Acute (humans)*	**0.1–2.5 mg/m³ [± OVA] (Fujimaki et al., 2004b)** [3] 0.5 mg/m³ [+ pollen] (Ezratty et al., 2007)			**Indeterminate**

Der f: *Dermatophagoides farina* (house dust mite); OVA: ovalbumin (major protein of chicken egg whites); both are immunogenic materials used to stimulate an allergic response.

Gray box = no data meeting the inclusion criteria were available.

Notes: Two studies with evidence that may inform the potential for formaldehyde exposure-induced inflammatory changes in the LRT are not captured in these tables, specifically a proteomics analysis of the BAL fluid after short-term exposure at ≥2.46 mg/m³ (Ahn et al., 2010) and an miRNA microarray study of gaseous paraformaldehyde exposure in a human lung cancer cell line with acute exposure to 1.23 mg/m³ (Rager et al., 2011). Swiecichowski et al. (1993) may include information from an earlier study interpreted to have been conducted in the same cohort of guinea pigs (Leikauf, 1992).

[a]Primarily, this reflects reporting of a statistically significant change; in rare instances where a *p* value was not given, changes are indicated if the authors discussed the change as a significant effect.

[b]Reported as 0.5% formaldehyde solution; concentration assumed to be >12.3 mg/m³ (Sadakane et al., 2002).

[c]Gene expression levels.

[d]These factors were not present at detectable levels regardless of treatment.

Changes in the blood and lymphoid organs

Although this mechanistic evaluation is focused on mechanisms underlying respiratory health effects, these effects can be influenced by changes in nonrespiratory tissue compartments, most notably the blood and lymphoid organs. The direction, magnitude and type of immune responses observed in the blood should not be assumed to represent immunological changes occurring in the airways, as responses can differ. The nonrespiratory changes most likely relevant to respiratory system health effects are immune-related changes because these could induce extrapulmonary signals (e.g., cellular; secreted factors) to travel through the blood to perfused regions of the respiratory tract. This section emphasizes changes in exposed humans, unlike the emphasis on experimental animal studies in the URT and LRT sections, because blood sampling in humans is more convenient than sampling from respiratory tissue compartments; thus, more human data are available for changes in the blood.

A number of studies, across different human and animal populations, spanning an array of formaldehyde exposure scenarios, have reported changes in blood cell counts. Although some of the specific changes vary across studies, taken together, the data provide *robust* evidence of an association between formaldehyde exposure and hematological effects. Although additional studies clarifying inconsistencies across the studies would be informative, several tentative patterns could be discerned. Interestingly, looking at the picture as a whole (see Figures A-31–A-32), the direction of some changes noted in the blood of individuals exposed to formaldehyde are contrary to the cellular changes noted in the respiratory tract. For example, data suggest (*slight*) or support (*moderate*) that total cells, neutrophils, and $CD8^+$ T cells are increased in the respiratory tract by formaldehyde exposure, while these same cells appear to be decreased in the blood (see Figure A-32). One potential explanation for this difference could involve recruitment of particular subsets of immuno-responsive cells from the circulation to the irritated and inflamed respiratory tract, as is observed with viral infections of the respiratory system (Levandowski et al., 1986); however, none of the identified human studies report data from both tissue compartments, and the animal data do not address such a hypothesis. It is plausible that this pattern could reflect species differences (i.e., LRT data are mostly from animal studies), but this possibility is considered unlikely given the blood data. As with investigations of the airways, very few studies tested mechanistic hypotheses for how formaldehyde exposure could affect blood immune cell counts. Despite this lack of information and variability in responses, the available data support a conclusion that formaldehyde exposure can modify immune system function in the blood across a range of concentrations and exposure durations.

One of the most consistent cellular changes observed across studies was a decrease in the total number of white blood cells (WBCs). This is a nonspecific finding, as WBCs encompass a spectrum of functional phenotypes, and this change may be driven by decreases in only one or several subpopulations. When looking more specifically at the WBCs, *moderate* evidence of $CD8^+$ T cell decreases following formaldehyde exposure is provided by several studies, together with a

corresponding increase in the ratio of $CD4^+/CD8^+$ T cells (see Table A-79). As mentioned previously, $CD8^+$ T cells comprise a heterogeneous cell population, which complicates interpretations regarding the potential impact of decreased numbers in peripheral blood. Depending on the specific stimuli, stimulated $CD8^+$ T cells can produce interferon-γ (IFN-γ) and inhibit production of IL-4 and immunoglobulin (i.e., IgE) responses (Holmes et al., 1997), or their phenotype can be driven towards production of excess IL-4, a situation hypothesized to be associated with atopic asthma (Lourenço et al., 2016). *Moderate* evidence provides support for increases in blood IL-4 (which was similarly increased in the LRT) and decreases in IFN-γ after formaldehyde exposure. A more complete understanding of the phenotype of the depleted $CD8^+$ T cells would be informative to ascertain whether these changes are related to the profile of secreted factors observed in the blood after formaldehyde exposure (see Figure A-31).[21]

Moderate evidence also indicates that formaldehyde exposure alters the number or percentage of B cells in the circulation. These cells produce antibodies upon stimulation with antigen (e.g., allergens) and contribute to airway hyperresponsiveness (Hamelmann et al., 1997). While this finding, along with *slight* evidence of increased antigenic markers, suggests potential for alteration of the adaptive immune response as a result of formaldehyde exposure, this observation alone is insufficient to indicate functional changes such as exposure-induced differences in clonal expansion and differentiation to antibody-producing cells, evidence of which would support a more convincing biological relationship.

Slight evidence suggests that neutrophils are also decreased in the blood by formaldehyde exposure. This could plausibly be explained by the suggestive (*slight)* findings of decreased lymphocyte and neutrophil chemoattractants in the blood and increased levels in the airways (possibly attracting blood neutrophils), suggesting that a gradient of these factors across tissue compartments may be induced and maintained as a result of formaldehyde exposure and, perhaps, sustained inflammation.

Finally, although variable across studies, several lines of evidence suggest a pattern of immune cell effects related to formaldehyde concentration, with stimulation at lower formaldehyde exposure levels and decreases at higher levels. This included changes in total T cells, NK cells, and IL-10 (and, perhaps, TNF-α). A complex relationship exists between IL-10, NK cells, and subsets of $CD4^+$ T cells (e.g., Th1 and Th2 cells), which direct the type of antibody responses; however, the specifics of this suggestive (*slight)* association with formaldehyde exposure remain to be elucidated. Many of these observations would benefit from additional, more specific studies on WBCs.

[21] Several studies examining the lineage and maturity of immune and non-immune cells in the bone marrow and other systemic tissues (e.g., blood; spleen) are not discussed in this section. Although it is possible that differences in the maturation phenotype of cells could indirectly contribute to the immune changes of interest to this section, such alterations would be expected to cause functional or other detectable changes in more apical mechanistic events relevant to immune responses in the respiratory system. Thus, this discussion focuses on those mechanistic events considered more directly relevant to these POE outcomes. Please see Section 1.3.3 of the Toxicological Review for a discussion of these cell lineage and maturation markers in the context of lymphohematopoietic cancer MOA.

Red blood cell (RBC) counts were decreased in both human and animal studies (*moderate* evidence), generally at formaldehyde concentrations above 0.5 mg/m^3. *Slight* data exist to suggest that platelets may also be decreased, which could plausibly be related to the single, low confidence animal study that reported increased megakaryocytes (cells that produce platelets) in the bone marrow (Zhang et al., 2013). The relevance of these changes to respiratory system health effects is unknown. It is plausible that sustained increases in oxidative stress (which has been observed in the blood and, to a lesser extent, other lymphoid tissues) and/or other soluble factors in the blood resulting from airway inflammation could affect the viability of circulating erythrocytes and immune cells or the circulating precursors for these cells; however, no evidence exists to substantiate this hypothesis. An increased level of the circulating stress hormone, corticosterone (the major animal glucocorticoid; in humans, it is cortisol), with short-term, but not acute, formaldehyde exposure is also suggested by *slight* data. Persistent increases in circulating glucocorticoids can also negatively impact the function and health of circulating immune cells, causing immunosuppression of most cell types (O'Connor et al., 2000). However, these potential linkages have not been examined.

As with findings for WBC changes, antibody, or immunoglobulin (Ig), responses resulting from formaldehyde exposure are consistently altered, although the specific changes observed across studies provide a mixed picture. Much of the *moderate* evidence is based on animal sensitization models using the protein allergen ovalbumin, although the human data also indicate changes after exposure. In general, the variable evidence of formaldehyde-induced modification of humoral immunity in humans demonstrates different patterns of results depending on the population (e.g., children vs. adults), the duration of exposure, and the specific Ig measure (e.g., Ig isotype) across studies. The animal studies consistently report amplified responses with allergen stimulation and/or sensitization, although the pattern and magnitude of these effects appears to vary depending on the type of allergen and the sensitization protocol used. The Igs most relevant to the blood and respiratory tract are IgA (IgA1 and IgA2), IgE, IgM, and IgG (IgG1, IgG2, and IgG3; also, IgG4 in humans). No changes of note in IgA or IgM were identified across the available studies. *Slight* data suggest that formaldehyde exposure may cause elevated levels of IgE antibodies in certain exposure scenarios, including in exposed children; however, this finding should be interpreted with caution, as comparable studies did not observe effects, and explanations for this inconsistency are not available. IgEs are implicated in allergic hypersensitivity responses of the airways (Hamelmann et al., 1999), although they may not be essential for all hypersensitivity-related responses (e.g., intrinsic [nonallergic] asthma occurs in one-third of all adult patients; Knudsen et al., 2009, 10085865}. Despite the variability in models, several of the available studies consistently identified changes in antibodies of the IgG class (*moderate* evidence), including increases in IgGs specific to formaldehyde or antigens (e.g., allergens) to which the subjects had previously been exposed. IgGs are the most prevalent Ig in the serum of humans, and they are the only Ig that can be transferred to neonatal/infant circulation (i.e., by crossing the placenta; through

breast milk in animals) to influence immunity in offspring (Van de Perre, 2003). None of the included studies examined antibody titers or transferred immunity with developmental formaldehyde exposure (note: *not informative* studies from one lab: Maiellero et al., 2014, 2375218; Ibrahim et al., 2015, 2966347 reported immune-related effects of gestational formaldehyde exposure). While IgEs are most commonly associated with sensitization-related airway hyperresponsiveness to allergens, subclasses of IgGs also contribute to allergic responses; however, their exact role in the pathophysiology of airway disorders remains unclear [Hofmaier et al., 2014, 10085863; Williams et al., 2012, 10085864; (Bogaert et al., 2009). Overall, although a body of evidence indicates changes in antibody-mediated responses after formaldehyde exposure, particularly in regard to IgGs, an explanation for the variable pattern of changes in Igs (e.g., to formaldehyde alone or with coexposure to different types of antigens by specific Ig subclasses) does not exist, and the likely consequences of these changes are unknown.

Table A-80. Summary of changes in the blood and lymphoid organs as a result of formaldehyde exposure

Endpoint		Study-specific findings from "high or medium" or "low" confidence experiments	Summary of evidence (exposure duration)	Conclusion
Formaldehyde-Induced Antibody Response in the Blood				
Total IgE	High or Medium	*Human*: None *Animal*: No evidence suggesting changes (Fujimaki et al., 2004b): subchronic ≤2.46 mg/m^3	No changes in a subchronic mouse study at ≤2.46 mg/m^3	**Moderate** Altered antibody responses (basis below) Total *Moderate*↓: IgG [naïve subjects] *Slight* ↑: IgE [3 mg/m^3] IgA [6 mg/m^3] *Indeterminate*: IgM [mixed] FA-specific *Moderate* ↑: IgG [long-term] *Slight* ↑: IgE [children; long-term] *Indeterminate*: IgM or IgA Antigen-specific *Moderate* ↑: IgG [inhaled antigen] *Slight* ↑: IgE [certain scenarios] *Indeterminate*: IgM or IgA
	Low	*Human*: No evidence suggesting changes (Ohmichi et al., 2006; Erdei et al., 2003; Wantke et al., 2000; Palczynski et al., 1999; Wantke et al., 1996b): short-term ≤1.8 mg/m^3 (duration in Erdei unknown) *Animal*: Evidence of increases in mice, which were increased further by OVA (Wu et al., 2013; Jung et al., 2007): short-term ≥3 mg/m^3; evidence of no changes in mice by FA alone (Kim et al., 2013a ; Gu et al., 2008), although FA exacerbated HDM-induced IgE (Kim et al., 2013a): short-term 0.12–1.2 mg/m^3	Suggestive evidence of increased IgE in 2 short-term formalin studies in mice at ≥3 mg/m^3, but no evidence for changes in mice or humans at <2 mg/m^3	
Formaldehyde (FA)-Specific IgE	High or Medium	*Human*: Elevated in one study of children (Wantke et al., 1996a): years (assumed) at ≈0.06 compared to ≈0.03 mg/m^3 (unrelated to symptoms); N/C in adults (Kim et al., 1999): 4 yrs at 3.74 mg/m^3 *Animal*: None	Increased in a single long-term study of children at <0.1 mg/m^3; N/C in a single long-term study of adults at 3.74 mg/m^3	
	Low	*Human*: No evidence of changes across multiple studies in adults (Ohmichi et al., 2006; Zhou et al., 2005; Kim et al., 1999; Wantke et al., 1996b; Górski and Krakowiak, 1991; Thrasher et al., 1987): short-term (weeks) or long-term (years) at ≈0.1–3.74 mg/m^3; unclear in 2 long-term adult studies in which a small proportion of subjects did have FA-IgE (Dykewicz et al., 1991; Thrasher et al., 1990); one study noted slight increases with longer exposure (Wantke et al., 2000): 10 wk, not 5 wk, at 0.265 mg/m^3 *Animal*: Isotype unspecified- no change in guinea pigs with acute challenge (Lee et al., 1984) at 2.5 or 4.9 mg/m^3 after short term exposure to 7.4 or 12.3 mg/m^3 (note: no measures without formaldehyde)	No clear evidence of changes across multiple short-term and long-term studies in adults at ≤3.74 mg/m^3; no studies in children	

Table A-80. Summary of changes in the blood and lymphoid organs as a result of formaldehyde exposure (continued)

Endpoint	Study-specific findings from "high or medium" or "low" confidence experiments		Summary of evidence (exposure duration)	Conclusion
Antigen-Specific IgE (does not include FA-specific Ig)	High or Medium	*Human*: None	N/C in a single subchronic study with i.p. sensitization	
		Animal: N/C in OVA-IgE (Fujimaki et al., 2004b): 12 wks at 0.1–2.46 mg/m^3 (OVA i.p.)		
	Low	*Human*: None	Two mouse studies suggest formaldehyde can increase IgE specific to antigen at ≈≥1 mg/m^3, but this appears to be highly situational (e.g., dependent on duration and periodicity of formaldehyde exposure, and antigen type and administration route)	
		Animal: Increased OVA-specific IgE in mice in 2 studies—(Gu et al., 2008; Tarkowski and Gorski, 1995): 10 d at 2 mg/m^3 (but not 1 d/wk for 7 wk, or when OVA sensitization i.p.) and 5 wk at 0.98 mg/m^3 with i.p. OVA (but not ≤4 wk), respectively; however, N/C in mice in 3 studies: (Wu et al., 2013): 4 wk at 3 mg/m^3 (s.c. OVA sensitization), (Kim et al., 2013b): 0.2–1.23 mg/m^3 for 4 wk (dermal house dust mite, HDM, sensitization), and (Sadakane et al., 2002): 4 wk at 0.5% (i.p. Der f sensitization)		
Total IgG	High or Medium	*Human*: Decreased in a single study of exposed workers (Aydın et al., 2013): 7 yr at 0.264 mg/m^3	A single study in adult workers and another in male rats showed decreased IgG at 0.264 or ≥6.15 mg/m^3 with long-term or short-term exposure, but subclass not examined	
		Animal: Decreased total IgG in rats (Sapmaz et al., 2015): short-term at ≥6.15 mg/m^3		
	Low	*Human*: N/C in children at ≈0.007–0.07 mg/m^3 (Erdei et al., 2003): unknown duration (likely months-years)	Suggestive evidence based on increased IgG1 in 2 short-term mouse studies, but a third mouse study and a human study did not observe effects at <1 mg/m^3	
		Animal: IgG1 (N/C in IgG2a) increased by FA alone, whereas FA exacerbated IgG2a (N/C in IgG1) in atopic-prone mice (Kim et al., 2013b): short-term 0.25, not 1.2 mg/m^3; increased IgG1 and IgG3, but decreased IgG2a and 2b, in C57 mice (Jung et al., 2007) short-term ≥6.15 mg/m^3; N/C in IgG Balb/c mice (Gu et al., 2008): short-term <1 mg/m^3		
FA-Specific IgG	High or Medium	*Human*: Slight (<10%) increase in a single study of adults (Kim et al., 1999): yrs at 3.74 mg/m^3	Slightly increased in a single long-term study of adults at 3.74 mg/m^3; no studies in children	
		Animal: None		

Table A-80. Summary of changes in the blood and lymphoid organs as a result of formaldehyde exposure (continued)

Endpoint	Study-specific findings from "high or medium" or "low" confidence experiments		Summary of evidence (exposure duration)	Conclusion
	Low	*Human*: Increased in two studies (Thrasher et al., 1990; Thrasher et al., 1987) and unclear in 1 study in which 5/55 subjects did have FA-IgG (Dykewicz et al., 1991): [all 3 studies] years at <0.1–<1.0 mg/m^3; N/C in one study (Wantke et al., 2000): short-term at 0.265 mg/m^3	Suggestive of slight increases in adults with long-term exposure at <1 mg/m^3, but not with short-term exposure at higher levels; no studies in children	
		Animal: Isotype unspecified—no change in guinea pigs with acute challenge (Lee et al., 1984) at 2.5 or 4.9 mg/m^3 after short term exposure to 7.4 or 12.3 mg/m^3 (note: no measures without formaldehyde)		
Antigen-Specific IgG (does not include FA-specific Ig)	High or Medium	*Human*: None	Increased OVA-IgG1 in 1 short-term study in guinea pigs at 0.31 mg/m^3 with inhaled allergen, but not a longer mouse study using injected allergen	
		Animal: Increased OVA-specific IgG1 in guinea pigs (Riedel et al., 1996): 5 d at 0.31 mg/m^3 (inhaled OVA); questionable decrease (no dose-response) in OVA-IgG1 and OVA-IgG3 in mice (Fujimaki et al., 2004b): 12 wks at 0.49, but not 2.46 mg/m^3 (OVA i.p.; N/C in OVA-IgG2)		
	Low	*Human*: Increased IgG against 2 bacterial pathogens by linear regression in 3rd grade children with respiratory complaints (Erdei et al., 2003): <0.1 mg/m^3, unknown duration (likely years, home measures)	1 long-term study suggests increased IgG sensitization to an airway antigen by FA in children; multiple studies in mice and rats suggest that IgG sensitization does not occur when antigen sensitization occurs by injection	
		Animal: N/C in OVA-IgG or Der f-IgG1 in mice (Wu et al., 2013; Gu et al., 2008; Sadakane et al., 2002): up to 5 wk at 0.123–3 mg/m^3 or higher; N/C in IgG specific to vaccine antigens in rats (Holmstrom, 1989): 22 months at 15.5 mg/m^3. In all cases, s.c. or i.p. sensitization		
Total IgM or IgA	Hight or Medium	*Human*: Decreased IgM, N/C in IgA, in a study of exposed workers (Aydın et al., 2013): 7 yr at 0.26 mg/m^3	IgM, but not IgA, decreased in a single study in adult workers at 0.26 mg/m^3 with long-term exposure	
		Animal: Increased total IgM and IgA in rats (Sapmaz et al., 2015): short-term at ≥6.15 mg/m^3		
	Low	*Human*: No evidence of IgA or IgM changes (Erdei et al., 2003): duration unknown ≤0.1 mg/m^3	IgA increased in 1 short-term study at >6 mg/m^3; N/C in IgM in 2 studies	
		Animal: Increased IgA and N/C in IgM in C57 mice (Jung et al., 2007): short-term ≥6.15 mg/m^3		
FA-Specific IgM or IgA	High or Medium	*Human*: None	No evidence to evaluate	
		Animal: None		

Table A-80. Summary of changes in the blood and lymphoid organs as a result of formaldehyde exposure (continued)

Endpoint	Study-specific findings from "high or medium" or "low" confidence experiments		Summary of evidence (exposure duration)	Conclusion
	Low	*Human*: Unclear evidence in 1 long-term study in which a small proportion of subjects appear to have elevated FA-specific IgM (Thrasher et al., 1990): months–years at ≈0.1–1 mg/m^3	Evidence could not be interpreted	
		Animal: Isotype unspecified- no change in guinea pigs with acute challenge (Lee et al., 1984) at 2.5 or 4.9 mg/m^3 after short term exposure to 7.4 or 12.3 mg/m^3 (note: no measures without formaldehyde)		
Antigen-Specific IgM or IgA (does not include FA-specific Ig)	High or Medium	*Human*: None	No evidence to evaluate	
		Animal: None		
	Low	*Human*: N/C in airway pathogen bacteria-specific IgM or IgA in one study in children (Erdei et al., 2003): unknown duration (likely months to years) at <0.1 mg/m^3	The minimal data available suggest that formaldehyde does not alter these parameters	
		Animal: N/C in IgM specific to vaccine antigens in rats (Holmstrom et al., 1989a): 22 mos at 15.5 mg/m^3 (s.c. injection)		
Immune and Inflammation-Related Changes in the Blood				
[[See Table A-81 for Cellular and Cytokine Response in Blood]]				
Oxidative Stress	High or Medium	*Human*: Increased marker of lipid peroxidation in adult serum lymphocytes (Bono et al., 2010): likely months to years (assumed) at ≥0.066 mg/m^3; Increased F2-Isoprostanes (suggested as the best in vivo biomarker of lipid peroxidation) in urine (Romanazzi et al., 2013): 0.21 mg/m^3 chronic occupational (indirect), although smoking and formaldehyde were not additive, both were independently associated with ROS—Note: serum and urine IsoP measures are correlated (Rodrigo et al., 2007), suggesting that urine levels may reflect similar serum changes	Two studies in adults indicate elevated oxidative stress markers in blood at ≥0.066 mg/m^3 with long-term exposure. Given the uncertainty with concluding urine levels exhibit the same pattern of association as blood, 1 study contributes as indirect evidence	**Moderate ↑**
		Animal: None		
	Low	*Human*: Increased oxidative stress biomarkers (F2-Isoprostanes; malondialdehyde) in urine (Bellisario et al., 2016): ≈0.034 mg/m^3 work shift occupational (indirect; responses likely reflect short-term exposure)	Several studies in three species suggest increases in markers of oxidative stress with acute or short-	

Table A-80. Summary of changes in the blood and lymphoid organs as a result of formaldehyde exposure (continued)

Endpoint	Study-specific findings from "high or medium" or "low" confidence experiments		Summary of evidence (exposure duration)	Conclusion
		Animal: Increased oxidative stress markers in mice (Ye et al., 2013; Matsuoka et al., 2010): acute or short-term as low as 0.12 mg/m^3; increased markers and protein indicators in rats (Aydin et al., 2014; Im et al., 2006): short term at 6.48–12.3 mg/m^3, although 1 study with longer exposure observed a decrease in MDA, but decreased SDH in lymphocytes (Katsnelson et al., 2013): 10 wk at 12.8 mg/m^3; other indicators including decreased GSH (Katsnelson et al., 2013; Ye et al., 2013) and increased NO and SOD (Matsuoka et al., 2010) at ≥1 mg/m^3	term exposure, even at formaldehyde levels ≤1 mg/m^3; it is not clear whether and to what extent this persists with long-term exposure	
Circulating Stress Hormones	High or Medium	*Human*: None	Increased stress hormone at 3 mg/m^3 formaldehyde in a single rodent study with short-term, but not acute, exposure	**Slight ↑**
		Animal: Increased corticosterone in rats with short-term, but not acute, exposure (Sorg et al., 2001a): ≈3 mg/m^3		
	Low	*Human*: None	No evidence to evaluate	
		Animal: None		
Altered Immune Function	High or Medium	*Human*: None	No evidence to evaluate	**Indeterminate**
		Animal: None		
	Low	*Human*: Increased autoantibodies in adults (Thrasher et al., 1990): long-term at 0.06–0.95 mg/m^3	1 study in adults suggests that autoantibodies are elevated with low level, long-term exposure; somewhat in contrast, 1 mouse study suggests short-term high level exposure improves host response to bacteria	
		Animal: Improved cell-mediated immune response to bacteria challenge, but N/C against tumor challenge or delayed-type hypersensitivity response in mice (Dean et al., 1984): 3 wk at 18.5 mg/m^3; however, N/C in vitro measures of immune cell function.		
Changes in Other Immune-related tissues				
Cell counts in immune tissues (not including bone marrow)	High or Medium	*Human*: None	Suppression of CD8+ T cells in immune tissues (e.g., spleen) is indicated in one 8-wk mouse study, with indirect support from a second short-term mouse study, at around 2 mg/m^3 ; effects on CD4$^+$/CD8$^+$ ratio	**Moderate** (for ↓ CD8+ T cell response in spleen and thymus)
		Animal: Decreased CD8+ T cells and increased CD4$^+$/CD8$^+$ ratio in both thymus (immature immune cells) and spleen (mature immune cells) in male mice (Ma et al., 2020): Eight weeks of exposure at 2 mg/m^3; No change in splenic CD4$^+$/CD8$^+$ ratio in female mice (Fujimaki et al., 2004b): 12 wk at up to 2.46 mg/m^3; Increased splenic regulatory T cells (subset of CD4+) and indirect markers for		

Table A-80. Summary of changes in the blood and lymphoid organs as a result of formaldehyde exposure (continued)

Endpoint	Study-specific findings from "high or medium" or "low" confidence experiments		Summary of evidence (exposure duration)	Conclusion
		suppression of effector T cell (CD8+) activity in female mice (Park et al., 2020): short-term exposure at ≥1.38 mg/m^3	were mixed across 2 subchronic mouse studies	**Slight** NK cells (in spleen: ↑ at low level; ↓ at high level) **Indeterminate** for other cell counts
	Low	*Human*: None	Multiple short-term mouse studies suggest that overall splenic cell T and B cells are unchanged; however, 2 studies suggest that NK cells may be affected (1 study showed NK cells were stimulated at low formaldehyde levels, and another that high levels are inhibitory/toxic)	
		Animal: N/C in tissue weight, total cellularity or T or B cell counts in mice (Kim et al., 2013a; Gu et al., 2008; Dean et al., 1984); altered NK cell number and function was noted in mice, with one study showing decreases (Kim et al., 2013a): 2–3 wk at 12.3 mg/m^3, and another showing increases (Gu et al., 2008): 5 wk at up to 0.12 mg/m^3, and a third showing N/C in lymphocyte proliferation, functional parameters, IgM production, or NK cytotoxicity (Dean et al., 1984): 3 wk at 18.5 mg/m^3		
Splenic and Lymph Cytokines and other Markers	High or Medium	*Human*: None	No evidence to evaluate	**Slight** ↑ oxidative stress and cytokine production, especially in response to antigen
		Animal: None		
	Low	*Human*: None	1 short-term mouse study suggests increased oxidative stress at ≥1 mg/m^3, and another ↓ IL-13 at 0.25–1.23 mg/m^3, and 3 others suggest that the response (splenic or lymph) to antigen stimulation (and 1 study without stimulation), most notably increased IL-4, is exacerbated at ≥0.25 mg/m^3 formaldehyde	
		Animal: Spleen: ↑ oxidative stress markers in mice (Ye et al., 2013): 7 d at ≥1 mg/m^3); exaggerated IFNγ response (at 2.46 mg/m^3) of lymphocytes to LPS and ↑ MCP-1 response to OVA in mice (Fujimaki et al., 2004b): 12 wk at ≥0.49 mg/m^3; ↓ IL-13 (Kim et al., 2013a): short-term at 0.25–1.23 mg/m^3; with allergen (HDM), exacerbated ↑ in IL-4, IL-5, IL-13, and IL-17a, but ↓ IFNγ (Kim et al., 2013a): short-term at 0.25 or 1.23 mg/m^3; Lymph Nodes: ↑ IL-4 and IL-10 (and IL-12, slightly), but N/C in IFNγ in mice with sensitization (De Jong et al., 2009): 4 wk at 3.6 mg/m^3; thymus: ↑ IL-4 and IL-1B in mice (Jung et al., 2007): short-term (2 wk) at ≥0.5 mg/m^3		
Bone Marrow Cell Counts and Function	High or Medium	*Human*: None	No evidence to evaluate	**Indeterminate**
		Animal: ↑ bone marrow hyperplasia in rats (Kerns et al., 1983): 24 mos at 17.6 mg/m^3		
	Low	*Human*: None		

Table A-80. Summary of changes in the blood and lymphoid organs as a result of formaldehyde exposure (continued)

Endpoint	Study-specific findings from "high or medium" or "low" confidence experiments		Summary of evidence (exposure duration)	Conclusion
		Animal: In mice: N/C in cell counts or functional properties in mice (Dean et al., 1984): 3 wk at 18.5 mg/m^3 [Note: thymus measures also unchanged]; Bone marrow toxicity, impaired function, and decreased cell counts at excessive levels (Yu , 2014, 2347224; Yu, 2015, 2803931): short-term at ≥40 mg/m^3; increased megakaryocytes (Zhang et al., 2013): short-term at ≥0.5 mg/m^3	1 mouse study suggests BM megakaryocytes may be increased with short-term exposure at ≥0.5 mg/m^3; Total cell counts are unchanged with short-term exposure at ≤20 mg/m^3 in 2 mouse studies, while excessive levels appear to cause toxicity	
Bone Marrow Cytokines and other Markers	High or Medium	*Human*: None *Animal*: N/C in BM mRNAs or miRNAs in rats (Rager et al., 2014): short term at 2.46 mg/m^3	Indirect evidence suggests no changes at ≤2.46 mg/m^3	**Slight ↑** oxidative stress and inflammation
	Low	*Human*: *Animal*: ↑ indicators of oxidative stress in mice (Yu et al., 2015a; Yu et al., 2014b; Ye et al., 2013; Zhang et al., 2013): short-term at ≥0.5 mg/m^3; increased markers of cell death (caspase-3) and inflammation (↑ NFkB, TNFα, IL-1β) in mice (Yu et al., 2015a; Zhang et al., 2013): short-term at 3 and 20 mg/m^3, respectively; N/C in DNA or RNA measures of proliferation and health in rats (Dallas et al., 1987): subchronic at 0.62–18.5 mg/m^3	3 mouse studies suggest that oxidative stress is increased with short-term exposure, even at 0.5 mg/m^3. 1 short-term mouse study suggests the BM is damaged and inflamed, while 1 longer-term rat study suggests there is no damage	

Table A-81. Summary of changes in blood cell counts and immune factors as a result of formaldehyde exposure

Endpoint(s)			No changes observed (*high or medium confidence* experiments are bolded)		Significant[a] increases or decreases (*high or medium confidence* experiments are bolded)		Summary conclusion Clarifying notes
			Duration[b] (species)	*Concentration(s) [notes] (study)*	*Duration (species)[b]*	*Concentration(s) [notes] (study)*	
White blood cells (WBCs)	Total WBCs		***Years (humans)*** **0.87 mg/m³ (Lyapina et al., 2004)** ***Years (humans)*** **0.25 mg/m³ (Aydın et al., 2013)** ***Short term (mice)*** **≥9.23 mg/m³ (Morgan et al., 2017)** *Years (children)* ≈0.02 mg/m³ [yr assumed] 90767		***Years (humans)*** ***Short term (rats)*** *Years (humans)* *Unclear[c] (humans)* *Short term (mice)*	**↓ 1.6 mg/m³ (Bassig et al., 2016; Hosgood et al., 2013; Zhang et al., 2010)** **≥2.46 mg/m³ (Rager et al., 2014); [indirect]** ↓ ≤0.29 mg/m³ [mean levels] (Kuo et al., 1997) ↓ N/A[h] (≤1 mg/m³) [yrs, not months] (Thrasher et al., 1990) ↓ 0.5–3 mg/m³ (Zhang et al., 2013)	**Moderate ↓** [4] Possibly concentration- and/or duration-dependent, but this dependence is unclear
	Granulocytes	All	*Short term (mice)*	18.5 mg/m³ [WBC differentials[d]] (Dean et al., 1984)	***Years (humans)***	**↓ 1.6 mg/m³ (Bassig et al., 2016; Hosgood et al., 2013; Zhang et al., 2010)**	**Slight↓** most likely neutrophils at higher concentrations with short-term or longer exposure
		Neutrophils	***Years (humans)*** ***Short term (mice)*** *Years (children)* *Years (humans)* *Short term (mice)*	**0.25 mg/m³ (Aydın et al., 2013)** **≥9.23 mg/m³ (Morgan et al., 2017)** ≈0.02 mg/m³ [yr assumed] (Erdei et al., 2003) ≤0.29 mg/m³ [mean levels] (Kuo et al., 1997) 0.5–3 mg/m³ (Zhang et al., 2013)	***Years (humans)*** *Short term (rats)*	**↓ 0.87 mg/m³ [note: function, not counts, in workers with URT dysfunction] (Lyapina et al., 2004)** ↓ 13 mg/m³ (Katsnelson et al., 2013)	
		Eosinophils	***Short term (mice)*** *Years (children)* *Years (humans)*	**≥9.23 mg/m³ (Morgan et al., 2017)** ≈0.02 mg/m³ [yr assumed] (Erdei et al., 2003) ≤0.29 mg/m³ [mean levels] (Kuo et al., 1997)			

Endpoint(s)		No changes observed (*high or medium confidence* experiments are bolded)		Significant[a] increases or decreases (*high or medium confidence* experiments are bolded)		Summary conclusion Clarifying notes
		Duration[b] (species)	*Concentration(s) [notes] (study)*	*Duration (species)[b]*	*Concentration(s) [notes] (study)*	
	Basophils	*Years (humans)*	≤0.29 mg/m³ [mean levels] (Kuo et al., 1997)			
Lymphocytes	**All**	***Months (humans)*** **0.2–0.8 mg/m³ (Jia et al., 2014)** ***Short term (mice)*** **≥9.23 mg/m³ (Morgan et al., 2017)** *Years (children)* ≈0.02 mg/m³ [yr assumed] (Erdei et al., 2003) *Years (humans)* ≤0.29 mg/m³ [mean levels] (Kuo et al., 1997) *Weeks (humans)* 0.51 mg/m³ (Ying et al., 1999) *Unclear[c] (humans)* N/A[h] (≤1 mg/m³) [yrs vs. months] (Thrasher et al., 1990) *Short term (mice)* 18.5 mg/m³ [WBC differentials[e]] (Dean et al., 1984)	***Years (humans)*** **↓ 1.6 mg/m³ (Bassig et al., 2016; Hosgood et al., 2013; Zhang et al., 2010)** ***Years (humans)*** **↑ 0.25 mg/m³ (Aydın et al., 2013)** *Short term (mice)* ↓ 0.5–3 mg/m³ (Zhang et al., 2013) *Short term (rats)* ↑ 13 mg/m³ (Katsnelson et al., 2013)	**Indeterminate** multiple changes noted, but pattern is indiscernible
	B Cells	***Years (humans)*** **1.6 mg/m³ (Bassig et al., 2016; Hosgood et al., 2013; Zhang et al., 2010)** ***Years (humans)*** **0.25 mg/m³ (Aydın et al., 2013)** *Years (humans)* 0.09–0.68 mg/m³ (Thrasher et al., 1987)	***Years (humans)*** **↓ 0.36 [up to 0.69 peaks] mg/m³ (Costa et al., 2013)** ***Months (humans)*** **↑ 0.99 [up to 1.69 peaks] mg/m³ (Ye et al., 2005)** ***Months (humans)*** ***Years (humans)*** **↑ 0.2 and 0.8 mg/m³ (Jia et al., 2014)** *Unclear[c] (humans)* **↓ 0.47 [up to 3.94 peaks] mg/m³ (Costa et al., 2019)** *Weeks (humans)* ↑ N/A[h] (≤1 mg/m³) [yrs, not months] (Thrasher et al., 1990) ↑ 0.51 mg/m³ (Ying et al., 1999)	**Moderate** For altered number of B cells (direction of change may differ by exposure levels or duration)

Endpoint(s)			No changes observed (*high or medium confidence* experiments are bolded)		Significant[a] increases or decreases (*high or medium confidence* experiments are bolded)		Summary conclusion Clarifying notes
			Duration[b] (species)	*Concentration(s) [notes] (study)*	*Duration (species)[b]*	*Concentration(s) [notes] (study)*	
		T Cells (Total)	***Months (humans)*** *Unclear[c] (humans)*	**0.2–0.8 mg/m³ (**Jia et al., 2014**)** N/A[h] (≤1 mg/m³) [yrs vs. months] (Thrasher et al., 1990)	***Years (humans)*** ***Months (humans*** ***Years (humans)*** ***Years (humans)*** *Years (humans)* *Years (humans)* *Weeks (humans)* *Short term (rats)*	**↓ 1.6 mg/m³ (**Bassig et al., 2016; Hosgood et al., 2013; Zhang et al., 2010**)** **↓ 0.99 [up to 1.69 peaks] mg/m³ (**Ye et al., 2005**)** **↑ 0.36 [up to 0.69 peaks] mg/m³ (**Costa et al., 2013**)** **↑ 0.25 mg/m³ (**Aydın et al., 2013**)** ↓ 0.09–0.68 mg/m³ (Thrasher et al., 1987) ↓ 0.9 mg/m³ [indirect: apoptosis] (Jakab et al., 2010) ↓ 0.51 mg/m³ (Ying et al., 1999) ↑ 7.4 mg/m³ (Sandikci et al., 2007a, b)	**Slight** mixed results suggests concentration-dependence, with ↓ at higher levels (possibly ↑ at low levels) with months–years exposure
		T Cells (CD4⁺)	***Years (humans)*** ***Months (humans)*** ***Years (humans)*** ***Years (humans)*** ***Months (humans)***	**1.6 mg/m³ [↓ T_{reg}] (**Bassig et al., 2016; Hosgood et al., 2013; Zhang et al., 2010**)** **0.99 [up to 1.69 peaks] mg/m³ (**Ye et al., 2005**)** **0.47 [up to 3.94 peaks] mg/m³ (**Costa et al., 2019**)** **0.25 mg/m³ (**Aydın et al., 2013**)** **0.2–0.8 mg/m³ (**Jia et al., 2014**)**	***Years (humans)*** *Weeks (humans)*	**↑ 0.36 [up to 0.69 peaks] mg/m³ (**Costa et al., 2013**)** ↓ 0.51 mg/m³ (Ying et al., 1999)	**Indeterminate** data suggest N/C, but variable, considering also studies of spleen above, suggests effects may exist at CD4 subset level

Endpoint(s)	No changes observed (*high or medium confidence* experiments are bolded)		Significant[a] increases or decreases (*high or medium confidence* experiments are bolded)		Summary conclusion Clarifying notes
	Duration[b] (species)	*Concentration(s) [notes] (study)*	*Duration (species)[b]*	*Concentration(s) [notes] (study)*	
T Cells ($CD8^+$)	***Years (humans)*** **0.25 mg/m³ (Aydın et al., 2013)** ***Years (humans)*** **0.36 [up to 0.69 peaks] mg/m³ (Costa et al., 2013)** ***Months (humans)*** **0.2–0.8 mg/m³ (Jia et al., 2014)** [N/C CD4/CD8 ratio in 3 studies and (Thrasher et al., 1990)		***Years (humans)*** **↓ 1.6 mg/m³ (Hosgood et al., 2013; Zhang et al., 2010)** ***Months (humans)*** **↓ 0.99 [up to 1.69 peaks] mg/m³ (Ye et al., 2005)** ***Years (humans)*** **↑ 0.47 [up to 3.94 peaks] mg/m³ (Costa et al., 2019)** *Weeks (humans)* ↓ 0.51 mg/m³ (Ying et al., 1999)[↑ CD4/CD8 ratio in **all but one of these** studies]	**Moderate ↓ CD8 and ↑ CD4/CD8 ratio** likely related to concentration
NK Cells			***Years (humans)*** **↓ 0.36 [up to 0.69 peaks] mg/m³ (Costa et al., 2013)** ***Years (humans)*** **↓ 1.6 mg/m³ (Bassig et al., 2016; Hosgood et al., 2013; Zhang et al., 2010)** ***Years (humans)*** ***Months (humans)*** **↑ 0.25 mg/m³ (Aydın et al., 2013)** **↑ 0.2, but not at 0.8 mg/m³ (Jia et al., 2014)**	**Slight** mixed results suggest role of concentration similar to total T cell findings
Monocytes	***Years (humans)*** **1.6 mg/m³ (Bassig et al., 2016; Hosgood et al., 2013; Zhang et al., 2010)** ***Years (humans)*** ***Short term (mice)*** **0.25 mg/m³ (Aydın et al., 2013)** **≥9.23 mg/m³ (Morgan et al., 2017)**		*Years (children)* ↑ ≈0.02 mg/m³ [yr assumed] (Erdei et al., 2003) *Short term (mice)* ↓ 0.5, but not 3, mg/m³ (Zhang et al., 2013) *Short term (mice)* ↓ 18.5 mg/m³ (Dean et al., 1984)	**Indeterminate** data suggest N/C, at least in human adults
Red Blood Cells	***Years (humans)*** ***Short term (mice)*** **0.25 mg/m³ (Aydın et al., 2013)** **≥9.23 mg/m³ (Morgan et al., 2017)** *Years (children)* ≈0.02 mg/m³ [yr assumed] (Erdei et al., 2003) *Years (humans)* ≤0.29 mg/m³ [mean levels] (Kuo et al., 1997)		***Years (humans)*** **↓ 0.87 mg/m³ [note: duration] (Lyapina et al., 2004)** ***Years (humans)*** **↓ 1.6 mg/m³ (Hosgood et al., 2013; Zhang et al., 2010)** *Short term (mice)* ↓ 0.5–3 mg/m³ (Zhang et al., 2013)	**Moderate ↓[6]** suggests combined role of concentration and duration

Endpoint(s)			No changes observed (*high or medium confidence* experiments are bolded)		Significant[a] increases or decreases (*high or medium confidence* experiments are bolded)		Summary conclusion Clarifying notes
			Duration[b] *(species)*	*Concentration(s) [notes] (study)*	*Duration (species)*[b]	*Concentration(s) [notes] (study)*	
Platelets			***Years (humans)*** **0.87 mg/m³ (Lyapina et al., 2004)** ***Short term (mice)*** **≥9.23 mg/m³ (Morgan et al., 2017)** *Years (children)* ≈0.02 mg/m³ [yr assumed] (Erdei et al., 2003) *Years (humans)* ≤0.29 mg/m³ [mean levels] (Kuo et al., 1997)		***Years (humans)*** *Short term (mice)*	**↓ 1.6 mg/m³ (Bassig et al., 2016; Hosgood et al., 2013; Zhang et al., 2010)** ↑ 0.5–3 mg/m³ (Zhang et al., 2013)	**Slight ↓** [7] possible concentration dependence similar to above
Secreted factors and immune markers	**Primarily Th1-related**	**TNF-α**	***Years (humans)*** **1.8 [up to 6.9 peaks] mg/m³ (Seow et al., 2015)** ***Months (humans)*** **0.2–0.8 mg/m³ (Jia et al., 2014)**		***Years (humans)***	**↑ 0.25 mg/m³ (Aydın et al., 2013)**	**Slight ↑ TNF-α and C3**
		Complement	***Years (humans)***	**(C3, C4) 0.25 mg/m³ (Aydın et al., 2013)**	***Short term (rats)***	**↑ (C3) 6.15 mg/m³ (Sapmaz, 2015, 2993350)**	
		IFN-γ			***Months (humans)*** *Short term (rats)*	**↓ 0.8, but not 0.2, mg/m³ (Jia et al., 2014)** ↓ 6.2–12.3 mg/m³ (Im et al., 2006)	**Moderate ↓ IFN-γ**
	Primarily Th2-related	**IL-4**			***Months (humans)*** *Short term (rats)*	**↑ 0.8, but not 0.2, mg/m³ (Jia et al., 2014)** ↑ 6.2–12.3 mg/m³ (Im et al., 2006)	**Moderate ↑ IL-4**
		IL-10			***Years (humans)*** ***Months (humans)***	**↓ 1.8 mg/m³ [less strict 20% FDR] (Seow et al., 2015)** **↑ 0.2–0.8 mg/m³ (Jia et al., 2014)**	**Slight IL-10** ***Suggestive*** of concentration role similar to total T and NK cell findings
		IL-6	*Acute (mice)*	0.12 mg/m³ (Matsuoka et al., 2010)			**Inadequate IL-6**
	Chemo-attractants	**CXCL11 (IFNγ-related)**			***Years (humans)***	**↓ 1.8 mg/m³ [stringent 10% FDR] (Seow et al., 2015)**	**Slight ↓** chemoattractants (attracting neutrophils-IL-8, and lymphocytes-Cxcl11, Ccl17)
		CCL17 (Th2-related)					

Endpoint(s)			No changes observed (*high or medium confidence* experiments are bolded)		Significant[a] increases or decreases (*high or medium confidence* experiments are bolded)		Summary conclusion Clarifying notes
			Duration[b] (species)	*Concentration(s) [notes] (study)*	*Duration (species)[b]*	*Concentration(s) [notes] (study)*	
		IL-8 (neutrophils)			***Months (humans)***	**↓ 0.2–0.8 mg/m³ (Jia et al., 2014)**	
	Other	**Ta1**			*Unclear [3] (humans)*	↑ N/A[h] (≤1 mg/m³) [yrs, not months, change in antigen reactivity markers] (Thrasher et al., 1990)	**Indeterminate** (data suggest N/C in B cell activation markers)
		IL-2R					
		CD27 and CD30	***Years (humans)***	**1.6 mg/m3 (Bassig et al., 2016)**			

Der f: *Dermatophagoides farina* (house dust mite); OVA: ovalbumin (major protein of chicken egg whites); both are immunogenic materials used to stimulate an allergy-like response

Gray box = no data meeting the inclusion criteria were available.

Note: one study observing increased substance P and related changes in the serum (Fujimaki et al., 2004b) is primarily discussed in the context of changes in the URT and LRT.

[a]Primarily, this reflects reporting of a statistically significant change; in rare instances where a *p* value was not given, changes are indicated if the authors discussed the change as a significant effect.

[b]Human study exposure durations are indicated as "years," "months," "weeks," or "acute" and defined based on the anticipated exposure duration for the majority of the exposed population(s); these durations are interpreted to approximate animal study exposure durations of chronic (>1 year), subchronic (several months), short term (<30 days), and acute (1 day or less).

[c]The comparison presented by Thrasher et al. (1990) reflects differences in exposure duration (years compared to weeks or months), but there appeared to be minimal difference in concentration.

[d]This finding (decreased total WBCs) is supported by 3 studies in humans evaluated by the NRC (2014) (Tong et al., 2007; Cheng et al., 2004; Tang and Zhang, 2003), but not evaluated in this analysis; additionally, this finding is supported by a study in mice (Yu et al., 2014b) and a study in rats (Brondeau et al., 1990), which are not included as they only tested formaldehyde levels ≥20 mg/m^3.

[e]Authors indicated no changes in "WBC differentials" other than decreased monocytes, but further details NR (Dean et al., 1984). This test was assumed to include basic granulocyte and lymphocyte counts.

[f]This finding (decreased erythrocytes) is supported by 1 study in humans evaluated by the NRC (2014) (Yang, 2007), but not evaluated in this analysis.

[g]This finding (decreased platelets) is supported by 2 studies in humans evaluated by the (2014) (Tong et al., 2007; Yang, 2007), but not evaluated in this analysis, and a mouse study testing excessive formaldehyde levels (Yu et al., 2014b).

[h]The exposure level is, in general, considered not applicable (N/A), as the comparison presented by Thrasher et al. (1990) reflected differences in exposure duration (i.e., years of exposure [Yr], as compared to weeks or months [Mo] of exposure), but there appeared to be minimal differences in concentration from the controls.

Consideration of mechanistic changes across tissue compartments

Several interesting relationships across tissue compartments are suggested:

- Evidence of increased oxidative stress, in particular, appears to be conserved across each of the evaluated tissue compartments. As soluble inflammatory signals can be transmitted across tissue boundaries with relative ease, it is plausible that these indications of an increased body burden of free radicals may be an indirect consequence of inflammatory changes that could be relatively restricted to the airways.

- Observations of increased eosinophils, and to a somewhat lesser extent, neutrophils, in both the URT and LRT, suggest that the inflammation of the airways caused by formaldehyde exposure is not restricted to the URT sites directly contacted by the majority of inhaled formaldehyde.

- Although some more subtle changes appear to occur in the LRT (e.g., inflammation; altered airway permeability), the data suggest that overt damage to the airway epithelium by formaldehyde exposure is limited primarily to the URT.

- Key features of several potential health hazards appear to involve mechanistic changes occurring within multiple tissue compartments, including decreased pulmonary function and allergic sensitization.

- Although many uncertainties remain, the instances of opposing immune-related responses in the airways compared to those in the blood suggest immunological communication and possible recruitment of cells from one compartment to another. One exception to this pattern was the consistent observation of increased IL-4 in both the LRT and blood. IL-4 is associated with driving CD4+ T cells towards a Th2 response (Kopf et al., 1993). The evidence specific to changes in CD4+ T cell populations in either compartment were inadequate, limiting interpretations of the significance of this finding.

- While many immune-cell-related changes were observed, some only occurred in specific exposure contexts. For example, neutrophil and monocyte increases in the LRT were observed only with allergen sensitization, while eosinophil increases were not observed in studies of exposure less than several weeks; changes in NK cells and other lymphocytes subsets appeared to vary depending on concentration, and some antibody responses depended on the antigen (e.g., allergen) type and administration methods. In addition, immune system studies after developmental exposure represent a significant data gap.

- In general, the evidence becomes less convincing with increasing removal from the point-of-first-contact for inhaled formaldehyde, with the highest confidence for effects in the URT, slightly less confidence for effects in the LRT and blood, and a general inability to draw conclusions regarding the potential for effects in lymphoid organs.

Plausibility of potential associations between mechanistic changes and respiratory system health effects

Figure A-34 illustrates one or more potential sequences of events from formaldehyde inhalation to apical outcomes (i.e., key hazard features) described in each of the respiratory system

health effects sections in the Toxicological Review. Each of these sequences was developed based on the most reliable mechanistic evidence (i.e., *robust* or *moderate* evidence was preferred) that can plausibly link an initial effect of inhaled formaldehyde to each of these key hazard features, and which have been demonstrated in formaldehyde-specific studies. Thus, these sequences do not represent all possible scenarios for which data exist (see Figures A-31 and A-32 for more comprehensive illustrations), and data not considered in this analysis (e.g., studies of chemicals closely related to formaldehyde) could identify additional initial alterations and mechanistic events, as well as more interim changes or relationships between many of the depicted mechanistic events. As such, this figure may not illustrate the most biologically pertinent sequence of events, but it does illustrate biologically plausible pathways of effects based on data specific to formaldehyde exposure. Thus, this is a pragmatic attempt to link early mechanistic events with apical endpoints, similar to the AOP conceptual framework (Villeneuve et al., 2014; Ankley et al., 2010)}. For each sequence, an interpretation regarding the likelihood of the presented sequence of events being a mechanism by which formaldehyde inhalation could cause respiratory system health effects is provided in the section below. As these interpretations are based on the robustness of the available evidence, they are primarily based on confidence in the individual studies and the consistency and coherence of observations across species and experimental paradigms. Other considerations outlined by Sir Bradford Hill (1965), including the magnitude and dose-dependency of the individual study findings, are discussed where the data are available, but these considerations generally had less of an impact on interpretations. This section references evidence conclusions from previous sections, as well as studies supporting biological understanding, but individual formaldehyde-specific studies are generally not referenced.

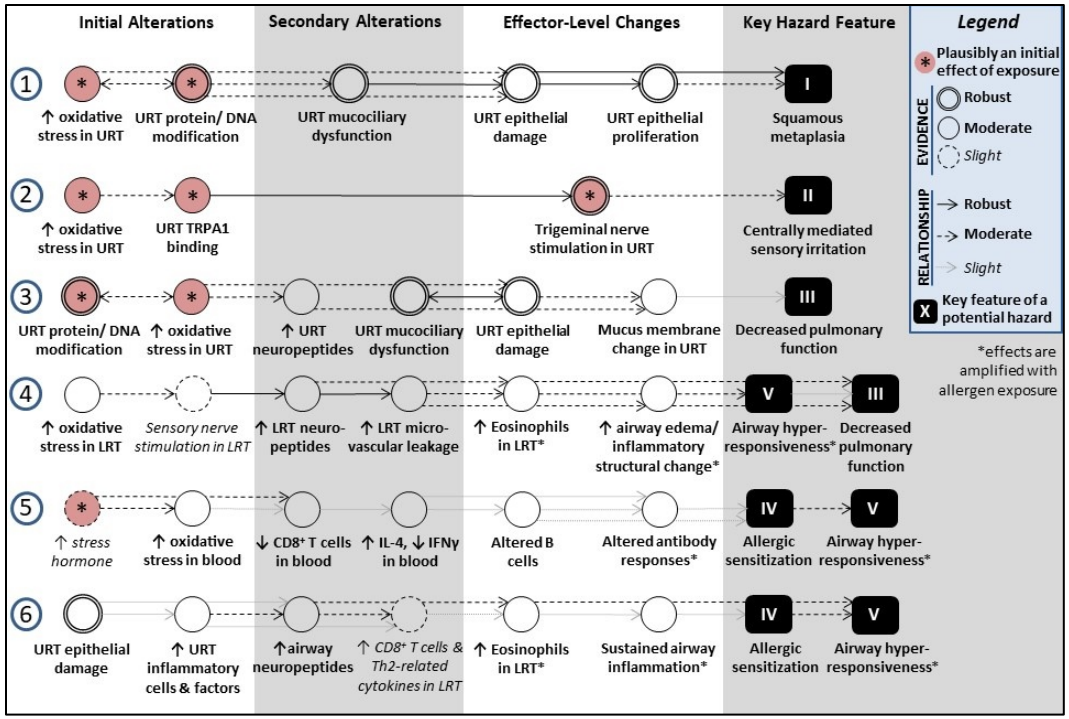


Figure A-34. Possible sequences of mechanistic events identified based on the most reliable evidence available.

This figures presents plausible mechanistic pathways illustrating the most reliable formaldehyde exposure-specific data (i.e., *robust* or *moderate* evidence was preferred) based on currently available information. The figure is organized by respiratory system health effect represented by key features of each hazard evaluated in the Toxicological Review. The pathways interpreted to most plausibly link possible initial effects of formaldehyde exposure to these apical events is presented, based on both the confidence in the relationships between events and confidence in the evidence for each of the linked mechanistic events. These pathways[22] are organized in a linear fashion from initial event(s) to key hazard feature(s), and each pathway is numbered, corresponding to the synthesis that follows. The mechanistic events are grouped into "initial events" and "secondary events" for endpoints that would be expected to occur earlier and later, respectively, along a sequential mechanistic progression. Generally, for the "initial" events, a preceding or precursor event other than a direct interaction with formaldehyde is unknown or has not been studied following formaldehyde exposure, or they have been described in previous pathways (e.g.,

[22] This approach draws some parallels to the AOP conceptual framework approach (Villeneuve et al., 2014; Ankley et al., 2010). As such, for those familiar with AOP terminology, it may be useful to think of the terms used herein according to related AOP terms (e.g., "plausible initial effects of exposure" and "initial alterations" relate to "molecular initiating events"; "mechanistic events" relate to "key events"; and "key hazard features" relate to "adverse outcomes").

see #6). "Effector-level changes" are those events that are most likely to be directly associated with the apical endpoint(s) of interest. The symbols, descriptors, and arrows are the same as those depicted in Figures A-31–A-32.

1) Respiratory tract pathology (squamous metaplasia) through epithelial cell damage

Interpretation: This is likely to be a major mechanism by which formaldehyde inhalation could cause squamous metaplasia.

Consistent with its known chemistry and reactivity, formaldehyde has been shown to react with DNA and other biological macromolecules at the point of first contact in the URT, where it also affects tissue redox capacity, presumably either through direct interactions with cellular macromolecules (e.g., lipids) or indirectly by impacting local tissue detoxification processes. These initial reactions have been shown to occur following acute and short-term exposure at concentrations <0.5 mg/m^3, and generally, the magnitude of these effects is expected to be driven largely by formaldehyde concentration and distribution. Distribution of formaldehyde-induced nasal lesions progresses to more posterior locations with chronic exposure; presumably, this represents changes in formaldehyde deposition, although this has not been tested. Additionally, studies have not been performed to address whether long-term exposure may overcome the body's capacity to regulate or restrict the magnitude of these changes. Elevated oxidative stress could directly lead to cytotoxic or subcytotoxic epithelial cell damage and/or dysfunction through the modification of cellular proteins and DNA. Because similar endogenous defense mechanisms (e.g., glutathione) are responsible for the detoxification of some free radicals and formaldehyde, persistent oxidative stress may make these cells more prone to damage directly resulting from formaldehyde and other inhaled agents. DNA-protein crosslinks (DPXs), which have been observed at formaldehyde concentrations ≥0.3 mg/m^3 (rats) or ≥0.9 mg/m^3 (rhesus monkeys) and durations ≥3 hours (see Appendix A.4), can lead to cellular damage if they are not repaired. Formaldehyde can modify the structure and function of the mucociliary apparatus, potentially as a result of covalent modification of soluble factors in the mucus (Morgan et al., 1984) or ciliary proteins (Hastie et al., 1990). Studies of the mucociliary apparatus following acute exposure provide evidence for a concentration threshold for functional effects, again highlighting the importance of formaldehyde concentration and distribution. In rats, DPXs and regions of mucociliary dysfunction have both been demonstrated to correlate with locations of subsequent respiratory tract pathology and cell proliferation in the anterior portions of the nasal mucosa following formaldehyde exposure. The resultant, potentially adaptive, effects on cellular proliferation (i.e., hyperplasia) are typically dose- and duration-dependent and localized to regions of mucociliary dysfunction and epithelial damage. Cellular proliferation may be initiated, at least in part, in response to formaldehyde exposures not associated with histopathological evidence of epithelial cell damage, since some studies report effects on proliferation at ≈1 mg/m^3. Direct and overt epithelial cell damage or death associated with squamous metaplasia is not typically observed until formaldehyde concentrations are above 2 mg/m^3. Squamous metaplasia is also localized initially to these high-flux, anterior regions, but these lesions increase in severity and advance to more posterior locations with longer exposure. Thus, although some early mechanistic events in this pathway are

expected to be highly dependent on formaldehyde concentration, the data supports a role for both exposure duration and concentration in the development of long-term lesions such as squamous metaplasia.

All of the events in this mechanism are based on *robust* or *moderate* evidence, with *robust* or *moderate* evidence for interactions between events, indicating that this mechanism is likely a major mechanism by which formaldehyde inhalation can cause squamous metaplasia. However, because modification of epithelial cell health and function in the URT can occur via multiple direct and indirect mechanisms following formaldehyde inhalation, which are expected to vary due to differences in both exposure duration and intensity, there are likely to be other important mechanisms by which formaldehyde exposure could cause respiratory tract pathology.

2) Sensory irritation through trigeminal nerve stimulation

Interpretation: This is likely to be the dominant mechanism by which formaldehyde inhalation could cause sensory irritation.

With distribution throughout the nasal mucosa, trigeminal nerve endings are well positioned for direct interactions with inhaled formaldehyde. Trigeminal nerve activation at unmyelinated C fibers occurs following acute formaldehyde exposure and the resultant physiological sensation of burning is known to be caused by afferent signaling to the CNS (Mackenzie et al., 1975). This afferent nerve activity has been demonstrated following formaldehyde inhalation. Based primarily on indirect evidence (e.g., ex vivo models), activation of the trigeminal nerve is probably at least partly dependent on direct activation of TRPA1 channels by formaldehyde (e.g., via binding). Further support for an "irritant receptor" response to formaldehyde exposure is provided by evidence of competitive inhibition of irritation caused by chlorine and acetaldehyde (Babiuk et al., 1985; Chang and Barrow, 1984). However, other direct actions of formaldehyde at trigeminal nerve endings (e.g., binding to other receptors; modification of ion balance; protein modification) are possible and some other potential pathway scenarios are suggested. In addition, oxidative stress, such as that elicited in the URT by formaldehyde exposure, is known to activate TRP channels (Bessac and Jordt, 2008), providing another plausible indirect mechanism. Based on the proposed sequence of events, sensory irritation would be expected to be highly variable across individuals due to differences in TRPA1 channel sensitivity or access of formaldehyde to TRPA1 channels (e.g., due to differences in airway structure, mucus production, or TRPA1 channel density). Studies of related chemicals suggest that human sensitivity may also be dependent on demographic factors such as age, sex (women appear to be more sensitive), and allergy status (Shusterman, 2007; Hummel and Livermore, 2002).

The threshold for activation of exposed rodent nerve endings has been reported at 0.31 mg/m^3 formaldehyde. The levels necessary for in vivo activation following acute exposure may be somewhat higher. Although trigeminal nerve activation may worsen with constant, repeated exposure to low levels of formaldehyde, as has been demonstrated for other chemicals

(Brand and Jacquot, 2002), constant exposure or high concentrations could conversely desensitize this response by excessively stimulatingf the (presumed) irritant receptors. The potential for sensory irritation to attenuate over time due to processes such as desensitization (e.g., via internalization of TRPA1 receptors) is unclear, particularly with long-term exposure. Indirect evidence suggesting either the presence of extremely sensitive individuals in the population or a role for the duration of exposure in eliciting this effect is provided from residential studies identifying symptoms associated with sensory irritation at levels as low as 0.1 mg/m^3 (e.g., e.g., Zhai et al., 2013; Liu et al., 1991; Hanrahan et al., 1984). Structural changes to the URT tissue (e.g., formaldehyde-induced modification of the epithelial cell layer altering accessibility of sensory nerve endings) and to the URT response of local immune cells (i.e., inflammatory cells may release mediators which can stimulate proliferation and/or sensitization of sensory nerve fibers (Carr and Undem, 2001) would be expected to be strong modifiers of this effect, introducing an exposure duration component to the concentration-dependence of receptor binding that is assumed for activation of TRPA1.

A strong biological understanding exists to identify the physiological sensation of sensory irritation as being related to stimulated sensory fibers of the trigeminal nerve. While the specific concentration and duration dependency of activation remain incomplete, based on the *robust* and *moderate* formaldehyde-specific evidence available to support activation of trigeminal nerve fibers and stimulation of TRPA1 receptors, respectively, along with a general lack of alternative explanations for chemical-induced sensory irritation, this mechanism is likely the dominant mechanism by which formaldehyde exposure can cause sensory irritation.

3) Decreased pulmonary function through URT epithelial damage

Interpretation: This is a possible mechanism by which formaldehyde inhalation could contribute to decreases in pulmonary function, but this is not a major pathway explaining this potential effect, and other changes are expected to be the primary drivers of any substantial functional changes.

Airway epithelial cells not only serve as a physical barrier to inhaled pathogens and antigens, they also participate in the regulation of airway inflammatory responses (Holgate et al., 1999). The demonstrated modification of the respiratory epithelium in the upper airways by formaldehyde exposure may affect pulmonary function through both physical, and humoral mechanisms, although definitive studies for the latter have not been conducted and such factors are generally tightly controlled and locally acting (e.g., Mayer and Dalpke, 2007, 10086279}. Modification to the URT epithelium by formaldehyde, particularly the observed effects on mucociliary function, is also likely to modify URT barrier and clearance processes, which could increase the impact of other inhaled antigens on pulmonary function; however, this possibility has not been well-studied. Physically, swelling of the mucus membrane has been observed in exposed humans at <1 mg/m^3 formaldehyde, and this is expected to be highly influenced by the underlying

respiratory status of the exposed individuals (e.g., allergy status; previous and/or current respiratory infections; etc.). This swelling can plausibly be linked to narrowing of the airways and impaired pulmonary function, although this linkage has not been explicitly demonstrated by corresponding effects in the LRT following formaldehyde exposure and it is unclear to what extent URT swelling would need to progress before effects on lung function were experienced. Morphological changes in the mucous membrane can be related to changes in mucus secretion and, possibly, epithelial cell proliferation (Reader et al., 2003), both of which are observed following formaldehyde exposure. Dysfunction of airway epithelial cells can also modify their release of humoral factors, which help to regulate airway smooth muscle contraction and immune cell responses. For example, epithelial cells can release neutral endopeptidase, which is the major metabolizing enzyme for tachykinins such as substance P and neurokinin A (Barnes, 1992), and they are known to produce situation-specific signals that can either promote or inhibit the activity of local immune cells, including dendritic cells, which contribute to airway remodeling (Lambrecht and Hammad, 2012). In these ways, modification of the function of URT epithelial cells by formaldehyde exposure might result, in an indirect manner, in changes in humoral factors that could reach the lower airways and lungs in minimal amounts. However, direct formaldehyde-specific examinations of such potential associations, including the requisite exposure parameters (e.g., levels), were not identified.

This sequence of events can plausibly link structural damage and dysfunction of the epithelium in the URT to potential decrements in pulmonary function. However, a large amount of missing information, particularly regarding LRT changes, is assumed, and evidence linking these formaldehyde-induced mechanistic events in the URT to changes in pulmonary function has not been reliably demonstrated. While these events might contribute to some minimal level of decrease in pulmonary function, the data are insufficient to identify this sequence of events as a major mechanism.

4) Airway hyperresponsiveness and/or decreased pulmonary function through LRT inflammatory changes resulting from sensory nerve activation

Interpretation: This is likely to be an incomplete mechanism by which formaldehyde inhalation could cause airway hyperresponsiveness and decreased pulmonary function, although whether certain events occur at low exposure levels is unclear.

Activation of airway sensory nerve endings is known to cause the release of neuropeptides, including substance P. Short-term formaldehyde exposure appears to cause increases in substance P, and perhaps other neuropeptides, in the lower airways. In addition, several lines of evidence identify potential substance P-related changes in the LRT that are at least partially dependent on TRP channel activation. As discussed previously, while certain, very rare human exposure scenarios might result in weak activation of the vagus nerve in proximal regions of the LRT (e.g., the trachea) due to direct interactions with formaldehyde, it is expected that the predominant

explanation (and that most relevant to interpretations) for activation remains unidentified and involves indirect pathway(s). One possible explanation involves indirect activation of LRT sensory nerve endings in association with the formaldehyde exposure-induced increases in LRT oxidative stress and/or inflammation, as certain electrophilic oxidative byproducts and inflammatory factors can stimulate TRPA1 channels (Andersson et al., 2008; Taylor-Clark et al., 2008). Alternatively, substance P could also be directly released from certain subsets of activated immune cells, including eosinophils (Joos et al., 2000), which are increased in the LRT, although this hypothesis has not been examined and may be somewhat less plausible, given the apparent discrepancy in the exposure duration required for substance P increases versus LRT eosinophil increases in the available studies. Regardless, any indirect pathway(s) would require prior modification of the LRT microenvironment after formaldehyde exposure through a separate, undefined mechanism.

Locally, substance P can cause vasodilation and leakage or constriction of airway smooth muscle, the latter of which appears to be enhanced in asthmatics (who also exhibit elevated substance P-immunoreactivity in airway nerves; Ollerenshaw et al., 1991, 10086342), all of which can contribute to airway narrowing or obstruction (Joos et al., 1995; Joos et al., 1994). It should be noted that airway obstruction typically requires much higher doses of agonist than does leakage (e.g., Yiamouyiannis, 1995, 3389495}. Formaldehyde-induced increases in substance P contribute to microvascular leakage in the LRT (i.e., trachea and main bronchi) following acute formaldehyde exposure, which has been observed at >1 mg/m^3. Specifically, although the effects of prolonged exposure were not examined, at higher formaldehyde levels (i.e., >10 mg/m^3) and with acute exposure, microvascular leakage was blocked by inhibition of the neurokinin 1 (NK_1) receptor, and perhaps also by inhibiting mast cell activation, but not by inhibition of histamine, cyclooxygenases, or bradykinin. Substance P is the preferred substrate for NK_1 receptors. Although activation of NK_1 receptors can contribute to structural changes in human airways, these receptors are more commonly associated with increases in airway inflammation (Schuiling et al., 1999). As introduced above, NK_1 receptors are also implicated in establishing the successful recruitment and adhesion of eosinophils and neutrophils to inflamed airways (Baluk et al., 1995), at which point these cells can release bronchoconstrictors. Thus, the increase in LRT eosinophils observed following formaldehyde exposure (and the *slight* evidence for increased neutrophils with allergen sensitization) could be related to elevated substance P. In addition, substance P itself can increase the responsiveness of the airways to bronchoconstrictors (Cheung C et al., 1994). Thus, either directly, or indirectly, the release of neuropeptides, presumably from stimulated sensory nerve endings, could result in airway hyperresponsivness. Perhaps relatedly, possible consequences of increased microvascular leakage and inflammation include airway edema and related structural changes, which have been reported following short-term formaldehyde exposures ranging from >0.3 to >3 mg/m^3 across studies, although these events have not been experimentally linked to sensory nerve stimulation or substance P signaling. Taken together, it is plausible that substance P-

mediated inflammatory alterations to the lower airways, were they of sufficient severity, could also lead to decreases in pulmonary function.

Several notable uncertainties exist for this plausible mechanistic pathway. As discussed above, an understanding of the sequence of events preceding the observed changes in the LRT remains largely incomplete. In addition, and perhaps most importantly, while most of the evidence is *moderate*, the data are based almost exclusively on acute or short-term experiments. Similarly, while evidence for some events at low formaldehyde levels (e.g., <1 mg/m^3) exists, some of the more convincing associations, including the requirement of NK_1 receptor activation for microvascular leakage, have only been tested at very high formaldehyde concentrations (e.g., >10 mg/m^3). Taken together, these limitations raise uncertainties for the relevance of this specific pathway to chronic, low-level exposure scenarios. Further, several important events related to this pathway have not been well studied. For example, the available studies have not examined the potential for sensory nerve activation to modify smooth muscle tone (e.g., regulation of contractile responses through the electrical activity; release of factors with direct action on smooth muscle cells, such as acetylcholine), and information does not exist to ascertain whether NK_2 receptor activation by neurokinin A, which can be a more potent bronchoconstrictor than substance P (Kraneveld et al., 2002), might be involved. Also, while substance P can stimulate mast cell degranulation and release of bronchoconstrictors such as histamine (Lilly et al., 1995, 10086423; Suzuki et al., 1995, 10086422), in vivo evidence of changes in mast cells was not identified. However, given the recruitment of other immune cells to the airways after formaldehyde exposure, an event that can be mediated by mast cells (Dawicki and Marshall, 2007), data on mast cells may represent critical information that is missing from the present analysis. Overall, based on the consistent *moderate* evidence for changes in the LRT that are commonly associated with changes in pulmonary function and airway responsiveness, this incomplete sequence of events is likely one of the mechanisms by which formaldehyde exposure could cause airway hyperresponsiveness and decreased pulmonary function. However, the pertinence of some or all of the components in this pathway with long-term, low-level formaldehyde exposure is unknown, and it is considered likely that other important mechanistic events would be identified with additional studies, particularly those testing longer exposure durations. It remains unclear how directly translatable this pathway, based largely on animal data, might be to interpreting complex human diseases such as asthma, and notable events thought to be important to the development or progression of asthma have not been observed.

5) Allergic sensitization and airway hyperreactivity through altered antibody-related responses in the blood

Interpretation: It is unclear whether this is a possible mechanism by which formaldehyde inhalation could cause these effects, as an understanding of the potential mechanistic relationships is incomplete.

Many reactive oxygen and nitrogen species (ROS, RNS) can be essential immunomodulatory signaling molecules. However, prolonged or excessive exposure to these factors can modify the structural and functional integrity of a wide range of cell and tissue types. Elevated indicators of oxidative stress have been identified in nearly all tissues examined following formaldehyde exposure, including the blood. In the blood of exposed humans, formaldehyde concentrations as low as 0.1 mg/m^3 have been shown to cause lipid peroxidation in peripheral immune cells, typically with prolonged exposure. The data are not available to demonstrate what might be causing this increase in free radicals, although factors released into the circulation as a result of pronounced or sustained airway inflammation would be expected to be capable of causing such an effect. Specifically, regarding the elevated corticosterone levels, which have been reported in rats exposed for several weeks to much higher formaldehyde levels (3 mg/m^3), an excess of glucocorticoids is typically associated with the inhibition of T cell cytokine secretion and function, although they may more specifically enhance the Th2 lineage and suppress the Th1 lineage (Taves and Ashwell, 2020; Elenkov, 2004). However, the varied roles for stress hormones (and free radicals) in the regulation of immune responses are complex (Glaser and Kiecolt-Glaser, 2005). Formaldehyde-specific studies examining the dynamics of this potential interplay were not identified.

Immunomodulatory effects of circulating stress hormones (and free radicals) could plausibly be associated with changes in circulating immune cells. As previously mentioned, although formaldehyde-induced changes in circulating immune cells were consistently observed, they varied in magnitude and direction across studies, suggesting a complex regulatory mechanism(s) for these effects. For example, decreases in CD8$^+$ T cells were primarily observed in the blood of individuals exposed to higher levels of formaldehyde (>0.5 mg/m^3), but not in studies testing lower exposure levels for comparable durations. CD8$^+$ T cells are composed of five subpopulations with numerous roles for both cell-mediated immunity and Th2-mediated allergies (Mittrücker et al., 2014). However, the majority of formaldehyde-specific studies evaluating T cell responses did not distinguish subpopulations of CD4$^+$ or CD8$^+$ T cells, since a number of these subpopulations have only recently been discovered, and some studies only assessed total T cells (see Table A-81). This complicates interpretations of these responses and raises the possibility that more consistency in changes across studies may exist for specific T cell subpopulations. Perhaps more importantly, the evidence for changes in CD4$^+$ T cells, which would be highly informative to this analysis as they are viewed as critical to the development of hypersensitivity (Cohn et al., 2004), was mixed and uninterpretable. Stimulated CD8$^+$ T cells produce IFN-γ, providing a plausible linkage between the decreases in CD8$^+$ T cells and the decrease in IFN-γ at >0.75 mg/m^3 formaldehyde in several studies. The observed increase in IL-4 at similar formaldehyde levels is more complicated, as its regulation is tightly controlled and likely to be mediated by multiple mechanisms. B cell proliferation and production of IgE and certain IgG subtypes is dependent on IL-4 and inhibited by IFN-γ (Paul et al., 1987), providing support for a relationship between these cytokine changes and altered IgG-related responses. The evidence of alterations in the number of B

cells, as well as the potential relationship between B cell levels and Ig levels, would benefit from additional study.

Understanding the regulation and function of IgE and IgG responses continues to evolve. IgE has a clear role in the development of allergic diseases that affect the airways, including allergic asthma, although IgE may not always be essential (e.g., in other types of asthma; in other allergic disorders). In contrast, IgG responses are poorly understood. While IgG may help to exacerbate IgE responses (e.g., patients with increases in both IgE and IgG are at greatest risk for developing allergic responses) and IgGs alone might induce allergic reactions to certain antigens (Wu and Zarrin, 2014; Williams et al., 2012; Finkelman, 2007), an excess of IgG antibodies can prevent IgE-mediated hypersensitivity and persons with increases in IgG alone are not typically at increased risk for allergic-related responses (Pandey, 2013; Williams et al., 2012; Strait et al., 2006). The evidence from formaldehyde-specific studies is insufficient to clarify whether IgE-mediated responses are involved (i.e., the evidence was considered *slight*, and was generally mixed and inconclusive), nor is it clear that changes in IgG are related to the development of sensitization or airway hyperresponsiveness. Further clarification of the observed IgG changes is also necessary, as some of the changes noted in response to formaldehyde exposure may depend on the duration of exposure or the specific IgG subtype examined. The antibody-related responses discussed herein have only been measured in the blood, as compared to samples that might be more directly informative to immune responses in the airways (e.g., nasal lavage or BAL). This is a notable data gap, given the somewhat disparate findings regarding immune cell counts in the airways and the blood. Overall, there are still critical uncertainties in the formaldehyde-specific antibody data.

In typical allergic disorders, changes in $CD4^+$ Th2 cells are present and are thought to play a prominent role, whereas $CD8^+$ T cell responses are generally lacking. Similarly, although IgG might contribute to allergic sensitization, the prototypical antibody response in allergy is thought to be largely driven by IgE. While it is possible that formaldehyde exposure may cause sensitization-related responses through a predominant IgG response rather than through IgE, the data demonstrating or proving such a linkage are not currently available. Overall, the available formaldehyde-specific studies do not provide information sufficient to disentangle the complex interplay between $CD4^+$ and $CD8^+$ T cells and B cells, regulatory cytokines such as IL-4, and the IgG and IgE responses that might underly the potential for formaldehyde to induce the interrelated immune effects of allergic sensitization and airway hyperresponsiveness.

Overall, the potential sequence(s) of events that may underly the observed changes in circulating antibodies remains poorly defined. Further, although a linkage between IgG responses and hypersensitivity is plausible, additional clarification is needed regarding the potential role for these types of changes in the pathogenesis of airway disease. Thus, based largely on an incomplete understanding of the necessity and ability of changes in IgG to induce these responses, and a lack of convincing formaldehyde-specific evidence demonstrating changes in IgE, it is unclear whether this is a possible mechanism by which formaldehyde exposure might cause these immune effects.

6) Airway hyperresponsiveness and allergic sensitization through airway eosinophilia and/or sustained airway inflammation

Interpretation: This is a likely a mechanism by which formaldehyde inhalation could cause airway hyperresponsiveness in those sensitized to allergens, although additional unidentified events are expected to contribute. It is also a possible mechanism by which formaldehyde inhalation could cause airway hyperresponsiveness in nonsensitized individuals. Whether this mechanism is useful for explaining the development of allergic sensitization is unclear.

A number of studies demonstrate that short-term formaldehyde exposure, and possibly longer-term exposure (the data are sparse), can cause an increase in eosinophils in both the upper and lower airways, particularly in animals sensitized to allergens. As previously mentioned, an understanding of how this recruitment occurs remains unclear. Although specific events proving a linkage have not been demonstrated, other formaldehyde-specific observations may be associated with this change. For example, airway epithelial cells, which are modified as a result of formaldehyde exposure, can release immuno-stimulatory factors, including the Th2 cytokines, IL-4 and IL-13, when exposed to allergens (Li et al., 1999). While changes in IL-4 have been noted in the LRT and could plausibly be related to altered epithelial cells mediating recruitment of eosinophils, the more important, and thus more convincing, evidence of such a linkage would involve increases in IL-3, IL-5, IL-13, GM-CSF, and/or eotaxin (Jacobsen et al., 2014; Trivedi and Lloyd, 2007; Wang et al., 2007a); however, the formaldehyde-specific evidence related to these latter factors is limited and generally inconsistent. Alternatively, eosinophil recruitment could be related to increased neuropeptide release from stimulated sensory nerve endings, as previously discussed. Bidirectional communication exists between sensory nerve endings and immune cells of the airways, and neuropeptide release can be enhanced by various cytokines and neurotrophins, including nerve growth factor (NGF) (Nockher and Renz, 2006). NGF, which can also induce mast cell degranulation and shift T cells towards a Th2 response (Mostafa, 2009; de Vries et al., 2001) and drive antigen-induced and tachykinin-mediated increases in inflammatory cells such as eosinophils (Quarcoo et al., 2004), may also be modified in the airways following formaldehyde exposure (Fujimaki et al., 2004b) (not shown in Figures A-31–A-32). Specifically regarding eosinophils, released neuropeptides such as substance P have been shown to prime eosinophils for chemotaxis by other factors such as leukotrienes or IL-5, and these neuropeptides can induce accumulated eosinophils to release factors associated with cellular activation, such as eosinophil cationic protein (Kraneveld and Nijkamp, 2001). Similar to the lack of evidence supporting a linkage with altered epithelial cell function, formaldehyde-specific data are not available to inform such potential linkages. Indirectly, neuropeptide release could also be associated with facilitating the recruitment of eosinophils to the airway by increasing the permeability of the microvasculature, although this evidence still fails to identify the immuno-attractant stimuli. Given the gaps in these linkages, it is likely that this sequence of events is incomplete. Of specific note, evidence of changes in $CD4^+$ Th2 cells in the LRT would be expected for each of these potential scenarios leading to

eosinophil recruitment, as these cells release factors such as IL-5 and are known to aid eosinophil recruitment in multiple experimental scenarios (Trivedi and Lloyd, 2007; Hogan et al., 1998).

Regardless of the mechanism of recruitment, the evidence indicates that airway eosinophils are increased by formaldehyde exposure, and activated eosinophils are known to affect airway contractile responses. Thus, even a short-lived increase in eosinophils could increase bronchoconstriction (e.g., through the release of mediators such as leukotrienes, major basic protein and M2 receptor antagonists, and through the activation of other immune cells such as mast cells and basophils, all of which can act on smooth muscle). However, the relationship of increased eosinophils to airway hyperresponsiveness or allergic sensitization to nonspecific stimuli is more complicated and depends on a combination of factors, many of which the formaldehyde-specific data do not address. For example, the longevity of this eosinophilic response following formaldehyde exposure, particularly in healthy individuals, remains unclear. Short-term eosinophil effects on pulmonary function with subsequent clearance of these cells from the airways would be unlikely to lead to prolonged hypersensitivity of the airways, which would be expected to involve persistent activation of these cells and continued production of pro-inflammatory mediators. A single animal study suggests that eosinophils persist with subchronic formaldehyde exposure at 2.3 mg/m^3 (but not at ≤0.5 mg/m^3) in animals sensitized to allergen (Fujimaki et al., 2004b), and other indirect evidence indicates that inflammation of the airways persists with long term formaldehyde exposure, particularly in those sensitized to allergens (see Table 1-80). However, it remains unknown whether these latter findings reflect the involvement of the populations of immune cells and secreted factors believed to be critical to the development of airway hyperresponsiveness. As previously described, the evidence examining the involvement of other important immunomodulatory events expected to affect airway responsiveness and allergic sensitization, including activation of basophils and mast cells, recruitment and/or development of a Th2 phenotype in CD4$^+$ T cells, evidence of remodeling[23] in the bronchi and/or alveoli, and changes in secreted factors known to affect smooth muscle reactivity, is generally *slight* or *inadequate*. These represent important data gaps.

Some experimental animal studies also report data suggesting increases in CD8$^+$ T cells in the LRT at very high levels of formaldehyde (>5 mg/m^3) with short term exposure. Similar to the observed LRT increases in eosinophils, the mechanism(s) mediating this recruitment to the airways is unknown, but likely to be downstream of formaldehyde-induced changes to epithelial cells and/or sensory nerve fibers. The observation of this change alongside the *moderate* evidence of decreases in CD8$^+$ T cells in the blood, generally suggesting a threshold for this effect around 0.5 mg/m^3, is of interest (note: similar trends in changes in other cells populations, including NK

[23] "Airway remodeling" has a specific meaning in human airway disease (see Bergeron, 2006, 10086904}. Several formaldehyde-specific animal studies defined the observed airway structural changes as remodeling (e.g., Wu et al., 2013; Liu et al., 2011; Qiao et al., 2009). Although the studies' data may relate to some aspects of airway remodeling, they are more generally described herein as inflammatory histologic changes to avoid misinterpretation.

cells, were also observed). Recruitment of lymphocytes to inflamed airways from the blood in response to acute insults is assumed for multiple respiratory disorders (Medoff et al., 2005) and has been demonstrated with different pathogenic stimuli, including exacerbation of asthma or COPD by rhinovirus infection (Mallia et al., 2014; Message et al., 2008). In these models, rhinovirus challenge generally causes an increase in BAL cells, including eosinophils and $CD8^+$ lymphocytes (and possibly neutrophils), while cell counts in the blood, including $CD4^+$ and $CD8^+$ T cells (and possibly NK cells) are decreased. In these types of studies, the specific relationship and magnitude of these changes appears to depend on the "dose" (e.g., viral load), as well as the sequence of pathology (e.g., viral challenge in symptomatic individuals). While the exact mechanisms underlying these complementary changes are unclear, hypotheses include modifications to epithelial cell function that leads to exaggerated immune responses in the absence of cytotoxicity (Gavala et al., 2013; Proud and Leigh, 2011). Thus, some of the observed airway inflammatory responses could be mediated through a sequence of events resulting from recruitment of certain immune cell populations from the blood to the airways, which may be directly relevant to changes observed in acutely challenged humans with airway disorders.

Overall, the evidence for persistent increases in airway immune cells and other immunomodulatory factors following formaldehyde exposure in individuals with prior allergen sensitization is interpreted as likely to represent an incomplete mechanism that could lead to airway hyperresponsiveness, as relevant observations have been reported after long-term exposure. However, the currently available data are insufficient to indicate this sequence of events as a likely mechanism for airway hyperresponsiveness in nonsensitized individuals. Owing to the lack of reliable formaldehyde-specific evidence demonstrating changes in IgE and other immunomodulatory factors assumed to be essential to the development of allergic responses, it is unclear whether this is a possible mechanism by which formaldehyde might cause allergic sensitization. Similarly, it remains unclear how useful this pathway might be to interpreting complex human diseases such as asthma. Additional studies are needed, particularly those employing long-term, low-level formaldehyde exposure.

Consideration of mechanistic pathways that may be associated with each potential respiratory system health effect

Several conclusions are suggested by the analyses of potential mechanistic pathways that might be associated with individual respiratory health effects, based on the most reliable formaldehyde-specific data:

- The confidence in the suggested mechanistic associations varies across the respiratory system health effects. While some uncertainties remain, important mechanistic events associated with sensory irritation, squamous metaplasia, and to a lesser extent, decreased pulmonary function, are supported by robust or moderate formaldehyde-specific data, and the relationships described are largely well-understood biological phenomena or have been demonstrated following formaldehyde exposure. Comparatively, the understanding of

mechanisms for potential immune effects is less complete. While moderate evidence exists for several mechanistic events that are likely to be involved in the development of airway hyperresponsiveness, the effect(s) at the point of contact that leads to these events is unclear. The mechanistic evidence describing the potential development of allergic sensitization is the most limited, as it includes slight evidence for several events, and the majority of the potential mechanistic relationships have not been experimentally validated and a clear scientific consensus regarding the relationships does not exist.

- The primary mechanism for sensory irritation is considered well understood, although it is based largely on acute or short-term exposures, and sensitivity is expected to vary between individuals. While studies clarifying the effects of tissue modification with longer term exposure in humans would be useful, it is likely that rodents exposed to ≈0.2 mg/m^3 formaldehyde under normal conditions would exhibit this effect. However, as exposure to formaldehyde appears to cause airway inflammation, which can increase the sensitivity and response magnitude of sensory nerve fibers, inflammation is viewed as a likely modifier of sensory irritation.

- At least one of the mechanisms by which formaldehyde exposure could cause squamous metaplasia is considered well understood, and it appears to depend on both exposure level and duration. Based on the pathway presented, these events are likely to occur at similar or slightly higher formaldehyde levels than those causing sensory irritation, and while cumulative tissue modifications with longer exposure or differences in human anatomy may increase sensitivity, the available experimental animal evidence suggests that pronounced effects leading to metaplasia are unlikely below 0.5 mg/m^3.

- Several contributing mechanistic pathways appear to impact pulmonary function, and the complex interactions within and across these pathways are expected to involve additional, unidentified factors. While some important mechanistic changes occur at low formaldehyde exposure levels (e.g., ≤0.2 mg/m^3 in rodents), data are not available to quantitatively relate these changes to decrements in pulmonary function. In addition, sensitivity is expected to be influenced by the respiratory health of exposed individuals. As with the mechanistic evidence supporting other health effects, much of the data is based on short term exposure. As exposure duration increases, and in the absence of potential compensatory mechanisms (which remains largely unexamined), amplification of these mechanistic events is expected.

- Given the lack of clear explanatory mechanisms for allergic sensitization, in particular, and uncertainties in data that may help to explain airway hyperresponsiveness, as well as an expectation of a large amount of important information that has not yet been identified in formaldehyde-specific studies, it is difficult to speculate on the exposure level- and duration-dependence of these potential pathways. However, some of the important events that may be involved (e.g., eosinophil increases) suggest a duration-dependence for the development of persistent changes in the sensitivity of the airways (note: transient hyperresponsiveness may be possible with short-term exposure), while other important data suggest that a concentration threshold likely exists in regard to critical changes in the cellular immune responses. Individual variability, including underlying respiratory health, is expected to be a significant modifier of these effects.

A.5.7. Nervous System Effects

Literature Search

A systematic evaluation of the literature database on studies examining the potential for noncancer nervous system effects in humans or animals in relation to formaldehyde exposure was initially conducted in 2012, with regular updates as described elsewhere (including a separate Systematic Evidence Map that updates the literature from 2017-2021 using parallel approaches; see Appendix F). The search strings used in specific databases are shown in Table A-82. Additional search strategies included:

- Review of reference lists in the articles identified through the full screening process.
- Review of reference lists in the 2010 draft Toxicological Review for Formaldehyde (U.S. EPA, 2010), the ATSDR toxicological profile of formaldehyde (ATSDR, 1999), and the NTP report on carcinogens background document for formaldehyde (NTP, 2010).
- "Snowball": review of references in review articles relating to formaldehyde and neurological effects (based on title and abstract screening), published in English, identified in the initial database search. For these articles, references were retrieved through Web of Science and added to the database via electronic export; manual review of references were conducted for the three reviews that were not found in Web of Science. Review articles that contained primary data were retained after full text screening.

This broad literature search was designed to identify studies in humans or animals that examined objective, apical effects on the nervous system, including structural, behavioral, chemical, and electrophysiological changes, as well as mechanistic studies informing potential biological associations between formaldehyde exposure and nervous system effects. Given the general lack of distribution of inhaled formaldehyde to the nervous system, likely in contrast to other routes of exposure and which complicates interpretations of direct interactions of formaldehyde with nervous system cells in tissue culture models, this search focused on inhalation exposure studies. Inclusion and exclusion criteria used in the screening steps are described in Table A-83.

The search and screening strategy, including exclusion categories applied and the number of articles excluded within each exclusion category, is summarized in Figure A-35. Although these noninhalation studies were considered for use, possibly to describe (in)consistent findings across exposure routes or as qualitative support for toxicological or mechanistic findings from inhalation studies, given the toxicokinetic uncertainties (e.g., possible differential distribution to the CNS), they ultimately were not included in the synthesis and were not considered further.

Table A-82. Summary of search terms for neurological effects

Database, Search Parameters	Terms
PubMed No date restriction	(formaldehyde [majr] OR paraformaldehyde) AND (neuron OR neurons OR neurono* OR neurolo* OR neuronal OR neurotox* OR neurophys* OR neurochem* OR neurotrans* OR neuropsych* OR neuropath* OR neuromusc* OR nerve OR nerves OR nervous OR electrophys* OR "evoked potential" OR *encephalog* OR encephalop* OR *sensory OR sensori* OR "central nervous system" OR CNS OR brain OR spine OR spinal OR spino* OR *axon* OR *synapt* OR *synaps* OR *myelin* OR dendrite* OR *behavior* OR learn* OR memory OR *motor OR *motion OR operant OR habituat* OR *coordination OR weakness OR righting OR reflex OR psychologic* OR mood OR sleep* OR visual OR audit* OR touch OR taste OR sound OR smell OR "pain sensitivity" OR nociception OR olfact* OR *glia* OR oligoden* OR astrocyte* OR balance OR sensation OR sensitization OR tremor* OR convuls* OR seizure* OR grip OR gait OR paralysis OR posture OR mobility OR rearing OR splay OR stereotypy OR conditioning OR avoidance OR approach OR neuropath* OR attenti* OR aggressi* OR arous*)
	NOT ("formalin test" OR "formaldehyde fixation" OR "formalin fixation" OR "formalin fixed" OR "formaldehyde fixed" OR "formalin-induced" OR "formalin-evoked") [Note: for quality control, ≈10% (50) of the 451 excluded article titles were scanned in PubMed: none were relevant]
Web of Science No date restriction Lemmatization "off"	SU= ("Anatomy & Morphology" OR "Behavioral Sciences" OR "Biochemistry & Molecular Biology" OR "Cell Biology" OR "Developmental Biology" OR "Life Sciences Biomedicine Other Topics" OR "Neurosciences & Neurology" OR Pathology OR Pediatrics OR Physiology OR "Public, Environmental & Occupational Health" OR "Reproductive Biology" OR "Research & Experimental Medicine" OR Toxicology OR "Veterinary Sciences" OR Psychology) AND TS= (formaldehyde OR paraformaldehyde OR formalin) AND TS= (neuron OR neurons OR neurono* OR neurolo* OR neuronal OR neurotox* OR neurophys* OR neurochem* OR neurotrans* OR neuropsych* OR neuropath* OR neuromusc* OR nerve OR nerves OR nervous OR electrophys* OR "evoked potential" OR *encephalog* OR encephalop* OR *sensory OR sensori* OR "central nervous system" OR CNS OR brain OR spine OR spinal OR spino* OR *axon* OR *synapt* OR *synaps* OR *myelin* OR dendrite* OR *behavior* OR learn* OR memory OR *motor OR *motion OR operant OR habituat* OR *coordination OR weakness OR righting OR reflex OR psychologic* OR mood OR sleep* OR visual OR audit* OR touch OR taste OR sound OR smell OR "pain sensitivity" OR nociception OR olfact* OR *glia* OR oligoden* OR astrocyte* OR balance OR sensation OR sensitization OR tremor* OR convuls* OR seizure* OR grip OR gait OR paralysis OR posture OR mobility OR rearing OR splay OR stereotypy OR conditioning OR avoidance OR approach OR neuropath* OR attenti* OR aggressi* OR arous*)
	NOT TS= ("formalin test" OR "formaldehyde fixation" OR "formalin fixation" OR "formalin fixed" OR "formaldehyde fixed" OR "formalin-induced" OR "formalin-evoked") [Note: for quality control, ≈2% (80) of the 3,825 excluded article titles were scanned in WoS: none were relevant].
ToxNet (Toxline and DART) No date restriction	formaldehyde AND (neurol* OR neurotox*) (including synonyms and CAS numbers, but excluding PubMed records)
TCATS2 Restricted to 01/01/2010 and newer	"formaldehyde" OR CAS Number: "50-00-0"

Table A-83. Inclusion and exclusion criteria for studies of nervous system effects

	Included	Excluded
Population	• Experimental animals • Humans	• Irrelevant species or matrix, including nonanimal species (e.g., bacteria) and studies of inorganic products
Exposure	• Quantified (e.g., levels; duration) exposure to inhaled formaldehyde in indoor air	• Not specific to formaldehyde (e.g., other chemicals) • No specific comparison to formaldehyde exposure (e.g., formaldehyde levels, duration, or similar in a study of exposure to a mixture)—NOTE: full text screening only • Outdoor air formaldehyde exposure—NOTE: full text screening only • Nonrelevant exposure paradigm (e.g., use as a pain inducer in nociception studies)
Comparison	• Inclusion of a comparison group (e.g., pre- or postexposure; no exposure; lower formaldehyde exposure level)	• Case reports (selected references used for illustration)
Outcome	• Nervous system effects that could indicate a hazard (e.g., behavioral, chemical, structural, or physiological) • Mechanistic studies examining aspects of nervous system function	• Subjective symptoms, including headache, fatigue, etc. • Effects other than noncancer nervous system effects, including carcinogenicity studies • Exposure or dosimetry studies • Use of formaldehyde in methods* (e.g., for fixation) • Processes related to endogenous formaldehyde
Other	• Original primary research article	• Not a unique, primary research article, including reviews, reports, commentaries, meeting abstracts, duplicates, or nonessential untranslated foreign language studies (these were determined to be off topic or unlikely to have a significant impact based on review of title, abstract, or figures). • Related to policy or current practice (e.g., risk assessment/management approaches or models)

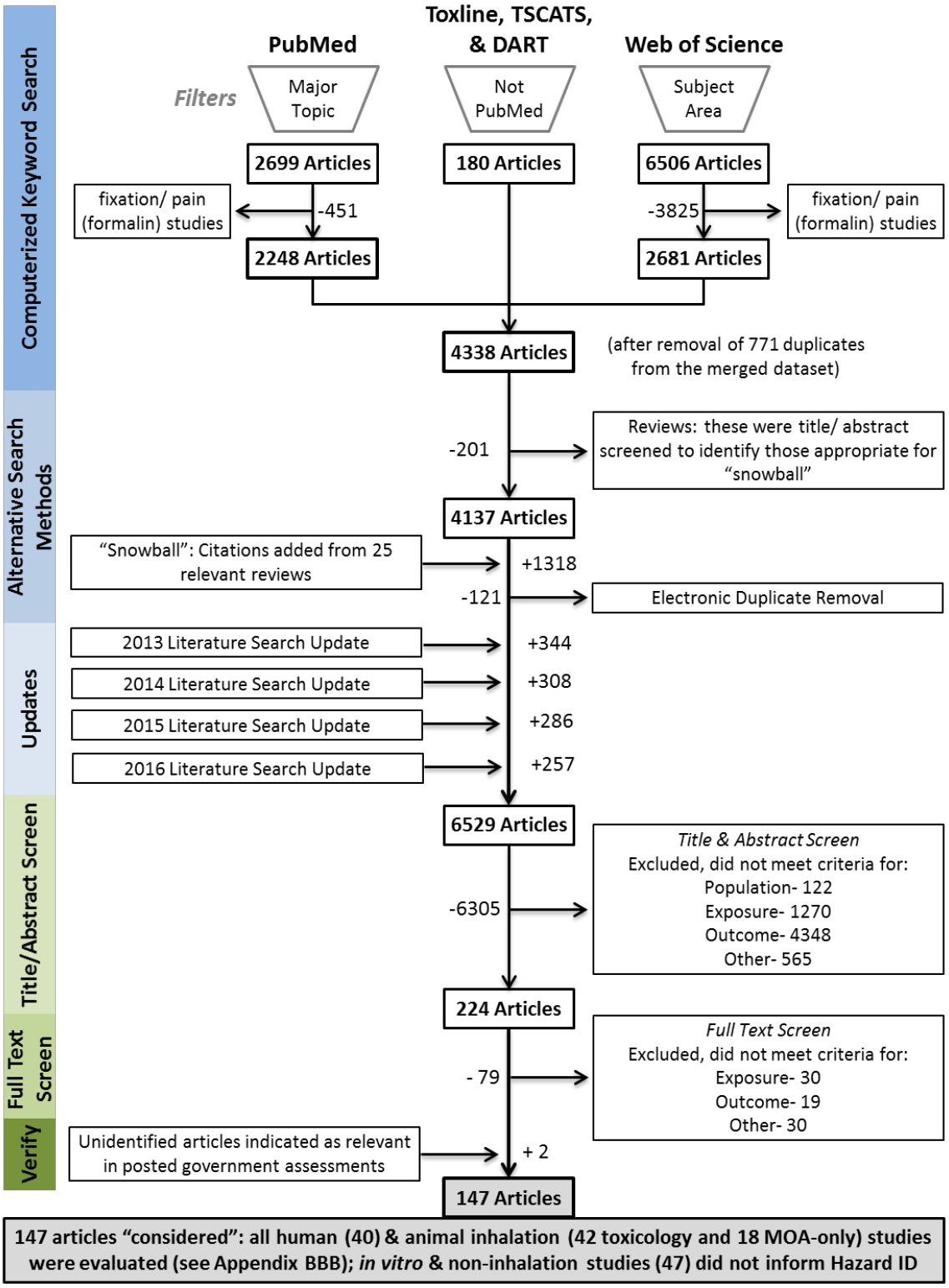


Figure A-35. Literature search documentation for sources of primary data pertaining to formaldehyde exposure and nervous system effects (reflects studies identified in searches conducted through September 2016).

Study Evaluations

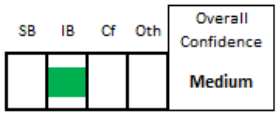
The studies identified in the literature search and screening process were evaluated using a systematic approach to identify strengths and limitations, and to rate the confidence in the results. EPA evaluated observational epidemiology studies of neurobehavioral effects and of risk of amyotrophic lateral sclerosis (ALS), controlled human exposure studies of neurobehavioral effects, and experimental animal inhalation exposure studies examining a variety of endpoints (e.g., learning and memory; motor activity, habituation, and anxiety; neuropathology). For controlled inhalation exposure studies (all chamber studies, including mechanistic studies), a separate evaluation was conducted examining details of the exposure protocol (formaldehyde administration and measurement (see Appendix A.5.1) that involved controlled formaldehyde inhalation was evaluated. The accompanying tables in this section document the evaluation. Studies are arranged alphabetically by first author within each table. The specific criteria for evaluation are described below.

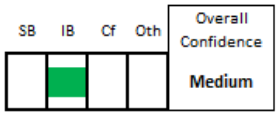
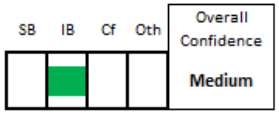
Human Observational Epidemiology Studies

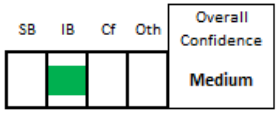
Amyotrophic lateral sclerosis is a rare neurodegenerative disorder of the motor neurons with an incidence in Western countries of 1–2 per 100,000 person-years (Ingre et al., 2015). Three of the studies of ALS evaluated ALS mortality which was not considered to be a limitation. Because the 5-year survival rate is low, mortality studies of ALS provide a good estimate for incidence of this disease. Because the disease is rare, the precision of risk estimates reported by these studies is a major limitation; the number of exposed cases for the case-control studies or total cases ascertained for the cohort studies generally was small. Established risk factors that should be considered as potential confounders are age, and sex. Smoking also has been associated with ALS in multiple studies. Family history also is a risk factor but would not likely be associated with formaldehyde exposure; therefore controlling for family history was not considered essential. While potential misclassification of exposure was another limitation for all of the studies, this was a particular concern for the general population studies, which collected exposure information using questionnaires (Fang et al., 2009; Weisskopf et al., 2009) or job-exposure matrices based on industry or occupation (Peters et al., 2017; Seals et al., 2017; Roberts et al., 2015). Fang et al. (2009) used a more detailed evaluation of exposure level and duration based on a structured occupational questionnaire and classification by industrial hygienists. Peters et al. (2017) and Seals et al. (2017) assigned individuals to exposure categories using the Nordic Occupational Cancer Study job exposure matrix which contained formaldehyde concentration data specific to either Sweden or Denmark; data on occupations over time were obtained from national censuses in Sweden (Peters et al., 2017) or the National Pension Fund in Demark (Seals et al., 2017). Roberts et al. (2015) used data from the National Longitudinal Study in the United States, which obtained information via a survey on the most recent occupation at the time subjects were enrolled; information on later occupations during follow-up was not captured.

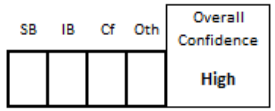
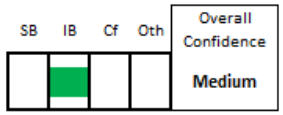
In addition to the general considerations for study evaluation, the observational and controlled human exposure studies that assessed a battery of neurobehavioral tests were evaluated with respect to the completeness and appropriateness of the battery of tests used, and the timing of their administration with respect to exposure.

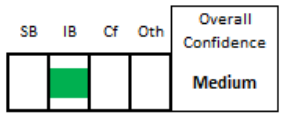
Table A-84. Evaluation of observational epidemiology studies of formaldehyde—neurological effects

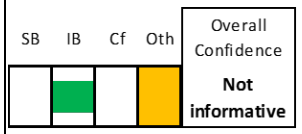
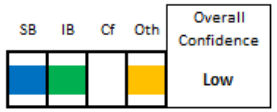
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Amyotrophic Lateral Sclerosis (ALS)							
Bellavia et al. (2021), (Denmark) Population-based nested case-control	Cancer cases, 1982–2009, from Seals et al. (2017) with data for several health factors and environmental risk factors previously linked with ALS. Controls, 100 per case matched on being alive on index date for case diagnosis, same birth year and sex. Excluded individuals with less than 5 ys wrork experience.	Occupational histories obtained from Danish Pension Fund databases. Used NOCCA (Nordic Occupational Cancer Study)- Danish JEM for periods 1960–74, 1975–84, and 1985 and after. Formaldehyde exposure metric was ever/never exposed. Anticipate exposure misclassification and large variation in prevalence and intensity of exposure across individuals. In men, correlations between formaldehyde, diesel exhaust and solvents were 0.22 and 0.41, respectively (Phi coefficients)	Danish National Patient Register, discharge diagnosis ICD-8 348.0 OR icd-10 G12.2. Case definition was 1st diagnoses on or after 1/1/1982–12/31/2009.	Evaluated diabetes, obesity, physical/ stress trauma, CVD (1977–2009) and lead, diesel exhaust and solvents	Selected joint predictors and interactions using boosted regression trees and Logic regression, which were included in a logistic regression model adjusting for age, SES, and geography. Model used a 3 yr lag.	1086 incident cancer cases, 677 exposed; 111,507 controls	Amyotrophic lateral sclerosis (incidence)  Uncertainty regarding exposure assessment. Adequacy of 3 yr lag is unknown.
Seals et al. (2017) (Denmark)	Registry-based case identification using the Danish National Patient	Occupational histories obtained from Danish Pension Fund	Danish National Patient Register,	Controls were matched to cases by age, sex	Conditional logistic regression	3650 incident cases,	Amyotrophic lateral sclerosis (incidence)

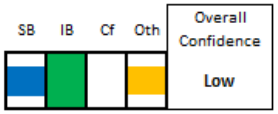
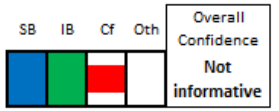
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Population-based case-control	Register, 1982–2009 (3,650 incident cases). Controls, 4 per case matched on sex, age, and no ALS diagnosis in Hospital Register as of index date obtained from Central Person Registry (All Denmark residents since 1968).	databases. Used NOCCA (Nordic Occupational Cancer Study)- Danish JEM for periods 1960–74, 1975–84, and 1985 and after. Inputs year and industry code and outputs prevalence of exposure for each job along with expected exposure level (ppm) in exposed. The JEM has not been validated to estimate levels. Cumulative expected exposure calculated (prevalence multiplied by expected level) summed over jobs and time (3 & 5 yr lags). Exposure misclassification expected.	discharge diagnosis ICD-8 348.0 OR icd-10 G12.2. Case definition was 1st diagnoses on or after 1/1/1982–12/31/2009.	and calendar date. Assessed SES (highest attained, 5 groups based on job title), marital status and residence. Other covariates were relative to 4th year before index year: whether worked on that year, years worked prior, hospital admission, # times admitted prior, # admissions, prior diagnoses used to construct Charlson Comorbidity Index. No information on smoking status	adjusted for age, sex, index date, SES, marital status and residence. In secondary analyses included other work variables, # hospital diagnoses, plus Charlson Comorbidity Index. Exposure metrics were dichotomous (ever exposed lagged 3 yrs), quantiles, and continuous	1,068 exposed; 14,600 controls	 Uncertainty regarding exposure assessment. Adequacy of 3 yr lag is unknown.
Fang et al. (2009) (United States) General population (case-control)	Sequential ALS cases recruited, 1993–1996, from 2 major referral centers in New England; eligibility criteria cases & controls: lived in New England at least 50% of	Occupational history by structured questionnaire; industry, occupation, frequency and duration; jobs held before ALS diagnosis	Diagnoses by board-certified specialists in motor neuron disease using World Federation of	Adjusted for age, sex, area of residence, smoking (ever/never), & education; no additional	Unconditional logistic regression models; linear trend with lifetime exposure days,	109 ALS cases (n=20 exposed) 253 controls	Amyotrophic lateral sclerosis (incidence) 

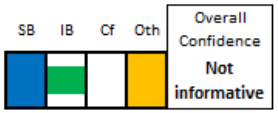
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	year, mentally competent, English speakers; 71% of eligible cases participated; controls by random telephone screening, frequency matched on sex, age (3 groups), & region; 76% of eligible (256 of 270 completed questionnaire).	or 2 yrs before interview (controls); formaldehyde-exposed occupations identified *a priori* by industrial hygienist; calculated life-time hours of exposure weighted by probability in specific jobs	Neurology El Escorial criteria	workplace exposures associated with ALS	probability, & weighted exposure duration (4 categories); effect modification by smoking; missing occupational data for 2/111 cases & 3/256 controls		Uncertainty regarding exposure assessment; small number of exposed cases
Peters et al. (2017) (Sweden) Nested case-control study	All Swedish births (1901–1970) and included in 1990 Swedish Population and Household census, N=5,763,437. Controls randomly selected (5 per case) from population alive on date of diagnosis, matched on birth year and sex. 25,100 controls.	Occupational history obtained from 1970, 1980, and 1990 census; included occupations listed ≥ 10 yrs prior to index date; occupational exposures assessed using Swedish version of JEM (Nordic Occupational Cancer Study), prevalence and level of exposure at specific calendar time. Exposure metric for dose response, prevalence multiplied by annual mean level for each occupation at time of census (mg/m^3),	Linkages to National Patient Register, primary or secondary diagnosis, ICD-9 335C or ICD-10 G12.2 (inpatient visits 1991-2010 and outpatient visits 2001–2010); follow-up to date of first visit, migration, death, or 12/31/2010. 5,010 cases	Addressed age and sex via matching, adjusted for education and evaluated 12 of >20 agents possibly associated with ALS. No adjustment for smoking status although restriction to blue collar workers and farmers may have partially addressed potential confounding	Conditional logistic regression, OR and 95% CI, adjusted for education and other 11 chemicals; restricted analyses to cases and controls with at least one occupation listed in any census and to blue-collar workers or farmers; sensitivity analysis	2,647 cases (n=323 exposed), 13,378 controls	Amyotrophic lateral sclerosis (incidence)  Uncertainty regarding exposure assessment

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		averaged across all censuses; dichotomized at median in controls			restricting to < 65 yrs at index date, age of retirement		
Pinkerton et al. (2013) (United States) Garment workers (cohort)	Cohort of garment workers (N=11,098) exposed for ≥ 3 mos at 3 facilities (late 1950s to early 1980s).	Monitoring in 1980s, geometric mean 0.15 ppm (GSD 1.9 ppm), constant levels across departments and facilities, year of first exposure (42% before 1963), time since 1st exposure (median 39.4 yrs) and exposure duration (median 3.3 yrs)	Vital status ascertained through 2008, ICD-10 G12.2, ICD-9 335.2, ICD-8 348.0, and ICD-7 356.1; ALS mortality is a good surrogate for ALS incidence	Adjusted for age, calendar time, sex, race; no information on smoking. Mortality for COPD and lung cancer in cohort was similar or greater than national rates suggesting possible bias away from null.	Life table analysis, excluded missing birth date (n-55), deaths (n=8), loss to follow-up prior to rate file begin date (n=13); SMRs and 95% CI	N = 11, 022, 414,313 person-years at risk; 8 ALS deaths	Amyotrophic lateral sclerosis (mortality)  Small number of cases. Confounding away from null not of concern because effect estimates were null.
Roberts et al. (2016) (United States) National Longitudinal Mortality Study. Occupational (cohort) Note: same laboratory and data handling procedures as	794,541 men and 674,694 women (recruitment date unclear, but study from 1973–2011) aged 25+ at recruitment (national). Follow-up time provided by participants. Internal comparison, participation unlikely to be influenced by knowledge of exposure and disease.	Self-reported at enrollment based on survey regarding last or most recent job. Exposure matrix constructed by industrial hygienists at the National Cancer Institute. Metrics included intensity and probability of exposure. Information on other	ALS Mortality (National Death Index from 1979–2011) as underlying cause; ICD-9 code 335.3 (specific for ALS) or ICD-10 code G12.2 (for all motor neuron diseases, of which ALS comprises the	Adjusted for education, race/ethnicity, and income (participants tended to be poorer, less educated, and less frequently non-Hispanic white. One sensitivity analysis among high probability, high exposure	Data handling and analysis as in Weisskopf et al. (2009) HRs provided for each exposure intensity and probability for men and women separately. Additional sensitivity analyses to evaluate validity of exposure and	472 deaths in men (100 exposed); 285 deaths in women (61 exposed)	Amyotrophic lateral sclerosis (mortality)  Uncertainty regarding exposure assessment, including the influence of duration, particularly in light of the use of a one-time survey at enrollment; very small number of exposed cases (n=2 in jobs with high probability and

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Weisskopf et al. (2009)		exposures not collected/reported.	overwhelming majority)	group (all funeral directors) included adjustment for smoking and military service.	outcome assignments and selection bias, included follow up restricted to 75 yrs or excluding first 5 yrs, age restricted to 35–75 or 50–75 yrs at enrollment, or restricted to those employed at enrollment. Did not provide or incorporate any data on duration.		intensity of formaldehyde exposure)
Weisskopf et al. (2009) (United States) American Cancer Society Cancer Prevention Study II. General population (cohort)	987,229 (414,493 men, 572,736 women) enrolled in 1982. National recruitment; no major illness at baseline, not missing age or sex data. Follow-up from 1989 through 2004. Internal comparison, participation unlikely to be influenced by knowledge of exposure and disease.	Self-reported, mailed questionnaire in 1982. Current or past regular exposure to formaldehyde and duration (yrs) (not specified, but likely in occupational settings). Data on 10 other types of chemicals and X-ray exposure also collected.	Mortality (National Death Index), underlying or contributing cause; ICD-9 (1989–1998) code 335.3 or ICD-10 (1999-2004) G12.2. (ALS represents > 98% of these categories)	Adjusted for age, sex, smoking, military service, education, alcohol, occupation (farmer, lab technician, machine assembler, programmer), vitamin E use, and the other chemical (and X-rays) exposures	Cox proportional hazards modeling, analyzed with and without approximately 1/3 who reported exposure but did not provide duration data (i.e., less likely to be truly exposed).	1,156 ALS deaths (36 exposed)	Amyotrophic lateral sclerosis (mortality)  Uncertainty regarding exposure assessment; small number of exposed cases

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
				assessed at baseline.			
Neurobehavioral tests and olfactory detection							
Broder et al. (1988c) (Canada; Toronto) Residences (household survey) Additional reference: Broder et al. (1988b)	Homes with UFFI insulation, within 60 miles of Toronto. 4,400 of 8,200 agreed to be contacted; 95% participated. Control homes randomly selected from streets adjacent to UFFI homes, 20% participated. Some demographic and symptom data allowed comparison with nonparticipants; similar neighborhood, demographics.	2-day samples in homes, 5 hr/d Median ppm Control 0.031 UFFI 0.038	Sense of smell threshold for pyridine; three control bottles (mineral oil only) plus 3 bottles with 0.00005, 0.008, and 0.012% pyridine. Replicate tests conducted. Variability and stability of test kits assessed. Participant blinded.	Detailed demographic data collected	Prevalence by group and Chi-square test.	1,726 from UFFI homes, 720 from control homes	Sense of smell  No appreciable difference in median exposure between groups
Kilburn et al. (1989b); Kilburn et al. (1987) (United States) Workers: histology technicians (survey)	Recruited from attendees (female) at annual histology technician conferences, 1982 and 1983. Participation rate not reported.	Self-reported hours per day (based on detection of odor)	Neuro-behavioral test battery (memory, cognition, spatial relation integration, dexterity, conceptual motor speed, balance,	Adjusted for age, number of cover slipped slides (for other solvent exposure), duration of smoking	Multiple regression. Coefficients and designation if $p < 0.05$ (no standard errors)	305	Neurobehavioral tests  Potential selection bias (could be influenced by perceived exposure and effects), limited detail presented in results

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
			reaction time); 1 hour				
Kilburn and Warshaw (1992) (United States) Workers: histology technicians (survey, multiple time points)	Recruited from attendees (female) at annual histology technician conferences, 1982, 1983, 1985, 1986, 1989. Participation rates not reported.	No information on intensity or frequency of exposure	Neuro-behavioral test battery (memory, cognition, pattern recognition, dexterity, decision making, motor speed, balance); 2–3 hrs	Considered age, sex, number of cover slipped slides (for other solvent exposure), yrs of exposure	For analysis of single (first) test per subject (n=350), reported as "not statistically significant." For longitudinal analysis (n=19), no decline in performance noted (formaldehyde exposure not explicitly analyzed).	19 with 4 tests, 299 with 2 or 3 tests, 350 with one test	Neurobehavioral tests  Potential selection bias, limited detail presented in results. Longitudinal analysis limited by sample size and did not specifically address formaldehyde exposure
Kilburn (2000) (United States, 6 states). Home or office exposure (survey)	Exposed (e.g., new mobile homes or renovated offices), experienced "adverse effects almost daily"; referent group randomly selected from voter registration rolls in 4 cities (location and participation rate not reported).	No exposure measures.	Neuro-behavioral test battery	Frequency matched by age and education	Mean ± SD percent prediction	20 exposed, 202 referents	Neurobehavioral tests  Likely selection of exposed based on symptoms; no exposure measures, limited covariate data.
Schenker et al. (1982) (United States)	People self-referred to occupational and environmental health clinic regarding health	Measured in 4 homes (protocol not described), ranged	Neurobehavior al battery	Not addressed	Prevalence	18 adults, 6 children (from 6 homes)	Neurobehavioral tests

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Residences (survey)	effects of formaldehyde insulation. No comparison group.	from 0.03 to 0.23 ppm					 Likely selection of exposed based on symptoms; limited exposure measures, no comparison group

Controlled Exposure Studies in Humans

Controlled human exposure studies were evaluated using a combination of criteria relevant to experimental animal studies (below) and criteria specific to studies in observational epidemiology studies.

Table A-85. Evaluation of human controlled exposure studies of formaldehyde – nervous system effects

Reference, setting, and design	Exposure assessment (quality descriptor and exposures)	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure) and confounding	Analysis and completeness of results	Size	Confidence
Andersen and Molhave (1983)	*Chamber type and analytical concentrations not provided;* **testing during exposure (distractibility likely contributes)** 4 d of exposure	*Endpoints limited*: sparse methods on conduct of partial neurobehavioral test battery	Exposure order by Latin square design; blinding not indicated	Comparisons appear to represent pooled sexes; **results data NR**	n=16	**Low**
Bach et al. (1990)	*Test article not defined* (inferred from (Andersen and Molhave, 1983))[c] **testing during exposure (distractibility likely contributes); acute (5.5 hr) exposure**	*Endpoints limited*: sparse methods on conduct of partial neurobehavioral tes battery	Occupation exposure group and controls from population registry (attempted matching by age, education, smoking prevalence but workers had higher smoking and lower education; details not reported); *Exposure order by balanced Latin square design; blinding not indicated*	**Results reporting incomplete & difficult to decipher**	n=61 males only	**Low**
Lang et al. (2008)	*Analytical concentrations achieved measured but not reported;* **testing immediately after exposure; study focus on irritation;** no indication of acclimation; recovery not examined (reaction time); 10 d of exposure	*Endpoints limited*: decision reaction time	Exposure order randomly assigned double blinded	Data= combined sexes; high variability in reaction time data	n=21 *≈20% attrition*	**Medium**

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Studies in Animals: Toxicological Studies

Hazard ID evaluations of chamber studies only encompass studies reporting results following in vivo inhalation exposures. Noninhalation exposures are expected to involve significant distribution of formaldehyde beyond the portal of entry (which is not observed to an appreciable extent following inhalation exposure).

Evaluation of experimental studies

As described in Appendix A.5.1., experimental animal studies were assigned the following confidence ratings: *high*, *medium*, or *low confidence*, and *not informative* based on expert judgement of each study's experimental details related to predefined criteria within five study feature categories. *Not informative* studies were designated based on the interpretation that the observed effect(s) are expected to have been driven by factors other than exposure to inhaled formaldehyde, or that the study did not provide a sufficient level of detail to evaluate the key methodological features or the nervous system-specific results. Due to the issues identified, the *not informative* experiments are not discussed in the Toxicological Review.

In addition to the general criteria discussed in Appendix A.5.1., considerations specific to the evaluation of potential nervous system effects were also evaluated. Due to the known neurotoxicity hazard of methanol, studies failing to use an appropriate test article were automatically assigned *low confidence* and, in an effort to avoid confusion with methanol's effects, if they evaluated high exposure levels (defined here as relying only on exposures > 10 mg/m^3) they were deemed to be *not informative*. Additional criteria included: consideration of the potential influence of irritation or changes in olfaction on behavioral measures (e.g., exposure during behavioral training was considered a limitation; a preference was given to behavioral studies with a period of latency between exposure and endpoint testing of 24 hours, or 2 hours at a minimum); blinding of the outcome assessors was preferred for subjective measures (e.g., slide evaluation; behavioral observations; etc.), although this was not necessarily considered a limitation for automated measures; a sample size of n = 10/group was preferred (n = 4 at a minimum); methods include a description of and a preference for endpoint evaluation procedures that are sensitive and specific for the detection of potential nervous system effects (see Table A-86 for additional details). Although studies with a longer exposure duration were considered to be most relevant to interpreting the lifetime neurotoxicity hazard of inhaled formaldehyde, nervous system effects studies of short term or even acute duration were not automatically considered to be less informative (i.e., exposure duration < 28 days was indicated as a minor limitation). This is somewhat in contrast to the interpretation of animal studies in other sections (e.g., respiratory tract pathology), and this reflects an understanding that neurotoxic effects from very brief exposures can oftentimes represent important health concerns. Additional considerations that might influence the interpretation of the usefulness of the studies during the hazard synthesis are noted, including limitations such as a short exposure duration or the use of only one test concentration or

concentration that are all too high or too low to provide a spectrum of the possible effects, as well as study strengths such as very large sample sizes or particularly robust endpoint protocols; however, this information typically did not affect the study evaluation decisions.

If the conduct of the experimental feature is considered to pose a substantial limitation that is likely to influence the study results, the cell is shaded gray; a "+" is used if potential issues were identified, but these are not expected to have a substantial influence on the interpretation of the experimental results; and a "++" denotes experimental features without limitations that are expected to influence the study results. Specific study details (or lack thereof) which highlight a limitation or uncertainty in answering each of the experimental feature criteria are noted in the cells. For those experimental features identified as having a substantial limitation likely to influence the study results, the relevant study details leading to this decision are bolded. Studies are organized according to the type of endpoint(s) evaluated, and then listed alphabetically.

Table A-86. Evaluation of controlled inhalation exposure studies examining nervous system in animals

	Experimental Feature Categories ***Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated***					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for hazard ID**
Criteria relevant to evaluating the experimental details within each experimental feature category	Exposure quality evaluations (see Appendix A.5.1) are summarized below; "++": robust; "+": adequate; and shaded box: poor; relevance of the tested exposure levels is discussed in the hazard synthesis	The species, sex, strain, and age are appropriate for the endpoint(s); sample size provides reasonable power to assess the endpoint(s); overt systemic toxicity is absent or not expected, or it is accounted for; group allocations can be inferred as appropriate	A study focus was nervous system effects; the exposure regimen is informative for the tested endpoint; latency from exposure to testing reduces the potential for irritation-driven responses Note: No guideline or GLP studies were identified [a]	The protocols used to assess the nervous system effects are sensitive for detecting an effect, complete, discriminating (i.e., specific for the response in question), and biologically sound; experimenter and sampling bias minimized	Statistical methods, group comparisons, and data presentation (including variability) are complete, appropriate, and discerning; selective reporting bias avoided	[Main limitations] Expert judgement based on conclusions from evaluation of the 5 experimental feature categories
Odorant or Irritant Detection/Effects						
(Apfelbach and Weiler, 1991)	**+** *Chamber type not specified*	**+** *N = 5* (exposed) or 10 (controls); *males only*	**Testing during exposure; controls not air-exposed in exposure chamber; possible continuous exposure** Note: 130 d exposure	**Training started 30d after exposures began (not clear if training ability prior to endpoint testing was affected)**	**++**	**Not informative** [Tested during exposure; missing controls; training during exposure]
(Wood and Coleman, 1995)	**++**	**+** *N*=8; *males only*	**Testing during exposure; each animal served as its own control (multiple exposures/animal);** *acute exposure (60 seconds on/off for ≈1hr)*	**++** Note: endpoint is not adverse (irritant detection)	**++** Note: statistical comparisons not possible	**N/A *** Olfactory detection/irritation response [Tested during acute exposure]

	Experimental Feature Categories ***Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated***					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for hazard ID**
Cursory Examinations in Long-Term Toxicity & Carcinogenicity Studies						
(Appelman et al., 1988)	++	+ N ≥ 10; *males only*	**Behaviors tested during exposure; study focus not nervous system-specific**; 1 yr study	+ *Endpoints limited*: cursory cage-side observations, gross pathology, & weight	**Results data NR; behavioral effects not quantified**	** [Tested during exposure; study focus not CNS; data NR]
(Coon et al., 1970)	+ *Multiple species exposed simultaneously*	+ *N=2* (i.e., dogs) to 15 (e.g., rats); *age & sex ratio/group not given* Note: multiple species tested	**Behaviors tested during exposure; study focus on overt toxicity and inflammation;** 90 d study	**Endpoints limited: cursory cage-side observations & brain sections "retained" (not clear if examined)**	**Results data NR; behavioral effects not quantified;** one death noted, but no cause provided	**Not informative** [Tested during exposure; limited endpoints; data NR]
(DHGC, 2010)	**Formalin (high concentration: methanol may drive responses)**	***N* = 3-6**	**Behaviors tested during exposure;** *acute exposure*	**Endpoints limited: cursory observations of behavior during exposure**	**Effects not quantified**	**Not informative** [High formalin levels; etc.]
(Kerns et al., 1983)[b]	++	++ *N*=10	**Behaviors appear to have been tested immediately after exposure; study focus on carcinogenicity** Note: based on a 2 yr GLP-compliant study ((Ciit), 1982), 3098; this was not noted in article	+ *Endpoints limited*: simple neurofunctional observations & gross pathology; methods provided in original CIIT (1982) study indicate *lack of observer blinding*	**Results data NR in published article; latency NR;** data in original CIIT (1982) study is qualitative (normal vs. abnormal) & is pooled across test battery endpoints	** [Tested immediately after exposure; study focus not CNS; data NR]
(Maronpot et al., 1986)	**Formalin**	++ *N*=10	**Behaviors tested during exposure; study design not nervous system-specific;** 13 wk study	+ *Endpoints limited*: cursory cage-side observations & gross pathology	**Results data NR; behavioral effects not quantified**	**Not informative** [Formalin; tested during exposure; study focus not CNS; etc.]

	Experimental Feature Categories ***Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated***					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for hazard ID**
(Morgan et al., 1986a)	**+** *Analytical concentrations not provided*	***N* = 3–6; males only**	**Behaviors tested during exposure; study design not nervous system-specific;** *acute exposure*	**Endpoints limited: cursory observations of distress during exposure**	**No quantified neurological effects**	**Not informative** [Formalin; small sample size; tested during exposure; etc.]
(Tobe et al., 1985a)	**Formalin** (Note: methanol control group included in the chronic study)	**+** *N* = 3–20 (depending on the experiment, endpoint & exposure group); *males only*	**Behaviors tested during exposure**; study design not nervous system-specific Note: studies of variable duration (up to 28 mos)	**+** *Endpoints limited*: cursory cage-side observations; gross pathology, brain wt. weight also performed in 28-month study	**Results details NR for many experiments & animals; behavioral effects not quantified;** multiple dead animals could not be examined for comparisons due to decomposition	****** [Formalin: controlled for some endpoints; tested during exposure; data NR]
(Woutersen et al., 1987)	**+** *Animals were housed in the inhalation chambers*	**++** *N*=40	**Behaviors tested during exposure**; study design not nervous system-specific Note: 13 wk study	**+** *Endpoints limited*: cursory cage-side observations, brain wt.	**Results data NR; behavioral effects not quantified**	****** [Tested during exposure; data NR]
Neuropathology						
(Aslan et al., 2006)	**++**	***N*= 3 litters (5 pups); males only; dam health during lactation & pup health not presented** Note: possible subset of Songur (2003) study; same animals as Sarsilmaz et al. (2007) study[e]	**+** *Unclear if potential litter bias was corrected* (although randomized treatment groups); *dams seemed to be co-exposed with pups* from PND 1–14 Note: 30 d of exposure	**++** Note: regional or hemisphere volume changes not verified by immunostaining, leaving interpretations unclear; sensitive stereology methods; random sampling indicated	**As presented, data do not account for potential litter effects (pup means presented)**	**Medium** [Small sample size; potential for litter effects]

	Experimental Feature Categories ***Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated***					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for hazard ID**
(Bian et al., 2012)	**Formalin (high concentration: methanol may drive responses)**	***N*= 3/endpoint/time point; males only; mild toxicity: decreased food intake (effect not quantified)**	**Controls not air-exposed in exposure chamber; all groups had anesthesia & antibiotic injections;** *exposures = 1 hr/d* Note: 90 d exposure; single exposure level	**+** Number of slides/animal not provided; relatively insensitive method for cell count quantification Note: blinding & other methods appropriate	**++**	**Not informative** [High formalin levels; etc.]
(Liu et al., 2010)	**Formalin (high concentration: methanol may drive effects)/static chamber**	**+** *Group size for staining not clear; males only*; groups determined by preexposure probe trial performance	**+** *Exposures only 30 min twice daily*; 28 d	**Potential sampling bias: details on blinding, slides/animal, etc. not provided; imaging specifics not provided and qualitative only**	**+** Hippocampal Nissl *staining not quantified*	**Not informative** [High formalin levels; etc.]
(Mei et al., 2016)	**Formalin**	**+** *N* = 8; *males only*	**+** *No comparisons to chamber or air exposure alone;* 8hr/d for 7 consecutive days	**Potential sampling bias: details on blinding, slides/animal, etc. not provided; qualitative only**	**No quantitative results (e.g., counts; severity scores; etc.)**	**Not informative** [formalin; potential sampling bias; no results quantification]
(Pitten et al., 2000)	**Formalin/static chamber**	**+** *N = 5*–8 Note: no changes in body weight were observed	**+** *Exposures only 10 min/d* for 90 da	**Potential sampling bias: details on blinding, slides/animal, etc. not provided; qualitative only**	**Results data NR**	****** [Formalin; potential sampling bias; data NR]
(Sarsilmaz et al., 2007)	**++**	***N*= 3 litters (5 pups); dam health during lactation & pup health not presented**;	**+** *Unclear if potential litter bias was corrected* (although randomized	**++** Note: regional or hemisphere volume changes not verified by	**As presented, data do not account for potential litter**	**Medium** [Small sample size; potential for litter effects]

	Experimental Feature Categories ***Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated***					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for hazard ID**
		males only[c] Note: possible subset of Songur (2003) study; same animals as Aslan et al. (2006) study[c]	treatment groups); *dams seemed to be co-exposed with pups* from PND 1–14; 30 d of exposure	immunostaining, leaving interpretations unclear; sensitive stereology methods; random sampling indicated	**effects (pup means presented)**	
(Songur et al., 2003)	**+** *Analytical concentrations not provided*	***N*= 6 pups (likely 3 litters); mild toxicity (body weight changes at 30 & 60 d, but not 90 d[d]);** males only	**+** *Unclear if potential litter bias corrected* (& not indicated as randomized); 30 d of exposure	**Cell counting methods do not detail how many slides/animal were examined (may be a single slide)**	**as presented, data do not account for potential litter effects (pup means presented)**	**Low** [Small sample size; potential for sampling bias and litter effects]
(Wang et al., 2014a)	**Mixture (formalin, benzene, toluene and xylene)/static chamber**	**++** *N* = 12 males/group Note: no changes in body weight were observed	**++** 2 hr/d exposure for subchronic (90 d)	**Relative, but not absolute (preferred), brain weights were reported; number of H&E samples NR** Note: both insensitive	**++**	**Not Informative** [Mixture exposure only; etc.]
Neural Sensitization-Related Responses						
(Sheveleva, 1971) (translation)	**Test article not defined (assumed to be formalin)**	**+** Use of mongrel white rats; *N*= 7 dams or 6 offspring/sex evaluated from 6 litters, so assumed 1 pup/sex/litter examined, but not specified; unclear why 7 dams vs. 6 offspring	**+** Latency between dam exposure and testing not provided: unclear if reflex bradypnea can influence these measures (e.g., reduced respiration leading to transiently reduced O_2 content in muscle tissue, causing reduced excitability); 4 hr/d exposures from GD1–19	**"Neuromuscular excitability" protocol specifics not provided (e.g., blinding; how assessed)**	**+** Statistical methods used were not specified; data appear to account for possible litter effects, but not clearly described	**Low** [Formalin; endpoint methods NR]

	Experimental Feature Categories ***Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated***					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for hazard ID**
(Sorg et al., 1996)	**Formalin (high concentration: methanol may drive responses)**	+ *N ≥ 4; females only*	**Potential high concentration irritation-related responses (that may affect odor discrimination in tasks involving exploration) were not measured;** *exposure 1 hr/d for 7 d*; Note: single exposure level	+ *Overall plus maze activity not provided*; Note: questionable human relevance of rodent sensitization responses	+ *Groups divided into high & low responders for presentation* of most endpoints & statistical comparisons; statistical comparisons NR for 1-month recovery data	**Not informative** [High formalin levels; etc.]
(Sorg et al., 1998)	+ *Chamber type not provided*; declining HCHO exposures across days	+ N= 15–24; *females only*	+ *Imprecise timing of assessment; unclear effect of prior cocaine exposure/handling on nociception (assumed to be minimal)* Note: 1 or 4 wk exposure; single exposure level	**Experimenter blinding not indicated;** methods for measuring vertical activity NR in cited reference Note: questionable human relevance	++	**Medium** [Blinding NR; limited methods description] Note: relevance of inescapable stress unclear
(Sorg and Hochstatter, 1999)	+ *Chamber type and analytical concentrations not provided*	+ *N = 4; females only* (conditioned fear) OR *N*= 8; *males only* (approach/avoidance)	**Possible effects on olfactory detection of conditioned odors by HCHO nasal effects; Approach/avoidance tested during exposure to formalin vapors** Note: 4 wk exposure; single exposure level	++ Note: questionable human relevance of rodent sensitization responses	**Effects without cocaine NR:** (unclear influence of prior cocaine exposure in conditioned fear responses)	**Low** [Unclear influence of changes in olfactory detection or prior cocaine exposure]

	Experimental Feature Categories ***Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated***					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for hazard ID**
(Sorg et al., 2001b)	**+** *Chamber type and analytical concentrations not provided*	**++** *N* = 7–8	**Testing during exposure;** exposures ≤ 4 wk Note: single exposure level	**+** *Methods for measuring vertical activity NR in cited reference (but automated using photocell counts)*	**++**	**Low** [Tested during exposure; limited methods reporting]
(Sorg et al., 2002)	**Formalin (likely high concentration- not quantified: methanol may drive responses); HCHO levels NR**	**+** *N* = 6–12	**Formalin used as an aversive stimulus- results more specific to cocaine; behaviors evaluated coincident with exposures;** *acute exposure*	**Tests involve odor detection & irritation-specific responses: could confound results** Note: questionable human relevance	**Specific effects of formaldehyde alone on behaviors NR;** some data presented with groups divided into high & low responders for statistical comparisons	**Not informative** [High formalin levels; etc.]
(Sorg et al., 2004)	**+** *Chamber type not specified*	**++** *N* = 7–8	**Possible effect on olfactory detection of conditioned odor by HCHO nasal effects; context testing prior to conditioned fear tests may cause order effects** Note: single exposure level; 4 wk exposure	**+** *Possible contribution of change in footshock sensitivity* not examined Note: questionable human relevance of rodent sensitization responses	**++**	**Low** [Unclear influence of changes in olfactory detection]
(Usanmaz et al., 2002)	**++**	**+** *N = 6; unexplained overt toxicity* (body weight decrease) with multiple exposures	**Observations immediately after exposure;** *acute (3 hr) or short-term (1–3 wk) exposure*	**Observations not blinded;** 5 min test duration; peripheral vs. central square crossings not measured, limiting interpretability	**++**	**Low** [Tested immediately after exposure; no blinding]
Motor Activity, Habituation, and Anxiety (& aggression)						

	Experimental Feature Categories ***Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated***					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for hazard ID**
(Boja et al., 1985)[e]	**+** *Analytical concentrations not provided*	**+** *N* = 8; *males only*	**Behaviors tested during exposure*; acute exposure (3 hr/d for 1–2 d)*;** timing of exposures (9–12 pm vs. 12–3 pm) may not have been same across groups Note: single exposure level	**Appropriateness of protocol for adult animals is questionable (methods designed for pups); "active" vs. "nonactive" endpoint readout is nonspecific**	**+** *Statistical comparisons to air-only exposure groups NR for all treatment groups; higher exposure groups data NR* and text suggests results are somewhat inconsistent	**Low** [Tested immediately after acute exposure; endpoint methods questionable]
(Katsnelson et al., 2013)	**Test article not defined (assumed to be formalin; high concentration: methanol may drive effects)**	**++** *N*= 12–15 females/group	**Testing indicated as immediately after exposure;** Note: subchronic (10 wk) exposure	Protocols not specified, although hole board test methods assumed to be conducted in a standard manner; **blinding not indicated**	**++**	**Not informative** [High levels of test article assumed to be formalin; irritation effects likely]
(Li et al., 2016)	**Formalin; static chambers**	+ *N* = 15 (inferred); *males only*	+ *Testing began ≈2 hr postexposure* Note: exposure 2 hr/d for 7 d	**Blinding not indicated for all tests except forced swim: of particular concern for nonautomated novel object testing; unclear impact of multiple tests in same animals** *(chosen test order may reduce impact); % open time in EPM does not include % closed time;* note: slight body weight loss 2.46 mg/m^3	**++**	**Low** [Formalin; endpoint evaluations fail to control for several important variables]

	Experimental Feature Categories ***Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated***					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for hazard ID**
(Liu et al., 2009a)	**Formalin (high concentration: methanol may drive effects)/static chamber**	+ *N* = 8; *males only*	+ *14 d exposure* Note: tested >24hr after exposure;	**Spontaneous locomotor activity was assessed subsequent to aggression tests, which may influence anxiety-related responses; blinding not indicated**	++	**Not informative** [High formalin levels; etc.]
(Malek et al., 2003a)	**Formalin**	++ *N*= 15/sex	+ *2 and 26 hr postexposure; acute: 2 hr*	+ *3 min test duration*; manual scoring (blinded); *peripheral vs. central square crossings not quantified*, limiting interpretability	+ Assuming data is SE, some statistical significance calls are questionable; *variability unclear*: SE reported is higher than SD for same parameters in 2003b	**Low** [Formalin]
(Malek et al., 2003b)	**Formalin**	++ *N*= 10/sex	+ *2 hr postexposure; acute: 2 hr*	+ *3 min test duration*; manual scoring (blinded); *peripheral vs. central square crossings not quantified*, limiting interpretability	++	**Low** [Formalin]
(Malek et al., 2004)	**Formalin**	+ *N* = 20; m*ales only*	+ *2 and 26 hr postexposure; acute; 2 hr*	+ *3 min test duration*; manual scoring (blinded)	++	**Low** [Formalin]

	Experimental Feature Categories ***Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated***					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for hazard ID**
(Senichenkova, 1991a) **(translation)**	**Test article not defined (assumed to be formalin)**	**Sex, *N*, & strain NR; could not be evaluated due to lack of reporting**	**+** *Unclear if litter bias corrected* Note: 4 hr/d exposures from GD1–19; single exposure level	**Open field protocol specifics not provided (e.g., blinding; manual vs. automated assessment of activity)**	**+** *Statistical methods NR*	**Not informative** [Test article assumed to be formalin; test animal and endpoint protocol details NR]
(Sheveleva, 1971) **(translation)**	**Test article not defined (assumed to be formalin)**	**+** *Mongrel white rats; N=6 offspring*/sex evaluated from 6 litters, so assumed 1 pup/sex/litter examined, but this was NR	**++** 4 hr/d exposures from GD1–19	**"Spontaneous mobility" protocol specifics not provided (e.g., blinding; manual vs. automated assessment of activity)**	**+** *Statistical methods NR*	**Low** [Test article assumed to be formalin; missing endpoint protocol details]
(Sorg et al., 1998)	**+** *Chamber type not provided; declining HCHO exposures across days*	**+** *N*= 15–24; *females only*	**+** *Imprecise timing of assessment* & **unclear effect of prior exposure to cocaine/handling on plus maze endpoints (assumed to be significant)** Note: 1 or 4 wk exposures; single exposure level	**Experimenter blinding not indicated (note: activity measures automated); overall plus maze activity not provided; unclear impact of saline injection, handling;** methods for measuring vertical activity NR in cited reference	**++**	Activity: **Medium** [Blinding NR; limited methods description; unclear impact of prior manipulations] Plus maze: **Low** [Blinding NR; limited methods description; overall activity NR; likely impact of prior testing]
(Sorg et al., 2001b)	**+** *Chamber type and analytical concentrations not provided*	**+** *N* = 6; *males only*	**+** No EEG/EMG sham controls and influence of 37% formalin irritation responses NR; *exposures ≤ 4 wk* Note: single exposure level	**No preformaldehyde sleep measures; sleep pattern methods NR** Note: questionable adversity of endpoints	**++**	**Low** [limited methods reporting; preformaldehyde comparisons NR] Note: questionable adversity

	Experimental Feature Categories ***Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated***					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for hazard ID**
(Usanmaz et al., 2002)	++	+ *Unexplained overt toxicity* (body weight decrease) with multiple exposures; *N = 6*	**Observations immediately after exposure;** *acute (3 hr) or short-term (1–3 wk) exposures*	**Observations not blinded;** 5 min test duration; peripheral vs. central square crossings not measured, limiting interpretability	++	**Low** [Tested immediately after exposure; lack of blinding]
Learning and Memory						
(Chonglei et al., 2012)	**Mixture (formalin, benzene, toluene and xylene)/static chamber**	+ *N= 5 males*/group	+ *Testing 30 min after exposure; 2 hr/d exposure for short term (10 d)*	**Path length or similar NR (contribution of motor effects not tested); visual cues NR; no blinding indicated**	++	**Not informative** [Mixture exposure; endpoint protocol deficiencies]
(Liao et al., 2010) **(translation)**	**Formalin/static chamber**	***N*=8: pooled sexes (*N*=4/sex); overt toxicity during exposure (e.g., listlessness; up to ≈30% decreased body weight gain), most likely from poor exposure quality, as only 0.5 mg/m^3 HCHO**	**Latency not provided (assumed that observations made immediately after exposure); no indication of correction for possible litter bias** Note: exposures 2hr/d for 28d	**Path length or similar NR (contribution of motor effects not tested); pool temperature, pool diameter, & platform size NR**; recovery time between escape latency trials not indicated; **no blinding indicated**	+ *Data= combined sexes* (test often displays sex differences)	**Not informative** [Formalin; overt toxicity; endpoint protocol deficiencies; etc.]
(Liu et al., 2010)	**Formalin (high concentration: methanol may drive effects)/static chamber**	+ *Males only*; treatment groups determined by performance in preexposure probe trials, but *unclear exactly how groups*	+ *Latency for all assessed time points unclear*, but appears that most had ≥24 hr habituation period between exposure and training/testing; *exposures*	++ Note: probe trials preexposure were comparable; cued trials conducted to rule out HCHO effects on vision	++	**Not informative** [High formalin levels; etc.]

	Experimental Feature Categories ***Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated***					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for hazard ID**
		were matched; Note: *N*=8-11	*only 30 min twice daily*; 28d exposure			
(LICM, 2008)	**Unspecified wood (possible co-exposures not tested)**	**+** *N = 5; males only*	**Training behaviors assessed 30 min postexposure and possible indirect effects of irritation on training may influence performance in the probe trial test;** *7 d exposure*	**+** *Path length or similar NR* (contribution of motor effects not tested); *no blinding indicated*	**+** *Comparisons across treatment groups NR for probe trial test*	**Low** [Likely mixture exposure; possible impact of irritation]
(Mei et al., 2016)	**Formalin**	**+** *N* = 8; *males only*	**+** *No comparisons to chamber or air exposure alone; testing 3 hr after exposure during training;* Note: 8 hr/d for 7 consecutive d	**Path length or similar NR (contribution of motor effects not tested); pool temperature, pool diameter, start positions & platform size NR; no blinding indicated (of concern, as not automated; note: cited references did not contain these details)**	**++**	**Low** [formalin; endpoint protocol reporting deficiencies; lack of blinding]
(Malek et al., 2003c)	**Formalin/static chamber**	**++** *N*= 15/sex/group; no changes in body weight were observed	**+** *Latency 2 hr postexposure*; *exposures for 2 hr/d for 10 d*	**Motor effects appear to drive some responses & were not tested (path length or similar NR); possible influence of changes in olfaction and/or vision not tested; blinding not indicated**	**+** *No ANOVA or trend tests* performed across the 4 groups (only pair-wise tests)	**Low** [Formalin; endpoint protocol deficiencies; no blinding]

	Experimental Feature Categories ***Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated***					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for hazard ID**
(Pitten et al., 2000)	**Formalin/static chamber**	**+** *N = 5*–8 Note: no changes in body weight were observed	**+** *22 hr postexposure; exposures only 10 min/d* Note: 90 d exposure	**+** *Possible influence of changes in olfaction and/or vision not tested; path length or similar NR*	**+** *Data= combined sexes* (test often displays sex differences)	**Low** [Formalin]
(Wang et al., 2014a)	**Mixture (formalin, benzene, toluene and xylene)/static chamber**	**+** *N = 6 males*/group Note: no changes in body weight were observed	**+** *Testing 30 min after exposure;* Note: 2 hr/d exposure for 49–90 d	**Path length or similar NR (contribution of motor effects not tested); visual cues NR; no blinding indicated**	**++**	**Not informative** [Mixture exposure; endpoint protocol deficiencies]
Nociception						
(Sorg et al., 1998)	**+** *Chamber type NR;* declining HCHO exposures across days	**+** *N*= 15–24; *females only*	**Imprecise timing of assessment following exposure**; unclear if cocaine or saline challenged Note: single exposure level; 1 or 4 wk exposures	**+** *Experimenter blinding not indicated*	**++**	**Medium** [Unclear exposure to testing latency]
Functional Observational Battery or Grip Strength						
(Chonglei et al., 2012)	**Mixture (formalin, benzene, toluene and xylene)/static chamber**	**+** *N= 5 males*/group	**+** *Unclear exposure to testing latency; 2 hr/d exposure for short term (10 d)*	**No description of grip strength protocol provided**	**++**	**Not informative** [Mixture exposure; endpoint protocol NR]
(Tepper et al., 1995)	**Carpet emission exposures: formaldehyde not primary exposure (BHT, toluene, etc.)**	***N*= 2 (nonexposed controls) or 4; males only**	**Behaviors tested immediately after exposure**	**++**	**Quantitative data NR for the majority of measures;** some measures presented as compared to preexposure or	**Not informative** [Mixture exposure; small sample; etc.]

	Experimental Feature Categories ***Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated***					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for hazard ID**
					summarized qualitatively	
(Wang et al., 2014a)	**Mixture (formalin, benzene, toluene and xylene)/static chamber**	**+** *N = 6 males*/group Note: no changes in body weight were observed	**+** *Unclear exposure to testing latency;* Note: 2 hr/d for 49–90 d	**+** *No blinding indicated;* Note: 5 s inter-trial delay and 3 trials/d	**++**	**Not informative** [Mixture exposure]
Electrophysiology (for Hazard; see below for MOA)						
(Bokina et al., 1976)	**Details of exposure were not provided**	**Details on test subjects were not provided**	**Details of study design were not provided**	**Details of endpoint measures were not provided**	**No quantitative comparisons to controls were performed**	**Not informative** [Experimental details NR]
Katsnelson, 2013, 1987924}	**Test article not defined (assumed to be formalin; high concentration: methanol may drive effects)**	**+** *N*= 12–15/group; *females only*	**+** *Testing indicated as immediately after exposure:* unclear if RB-related effects could affect these impulses Note: subchronic (10 wk) exposure	**++** Note: Citation for temporal summation of impulses protocol was provided	**++**	**Not informative** [High levels of test article assumed to be formalin]
Autonomic Effects (for Hazard; see below for usefulness for MOA)						
(Nalivaiko et al., 2003)	**Unregulated exposure without reporting of levels; no chamber** Note: paraformaldehyde	**+** *N = 6–13; males only*	**No nonexposed groups indicated (internal comparisons);** *acute exposure***;** All animals implanted with electrodes (duration before tests NR)	**+** *ECG implantation procedures NR* Note: endpoint not considered adverse	**++**	**Not informative** [Exposure levels NR and unregulated; etc.]
(Tani et al., 1986)	**Formalin (high concentration: methanol may drive responses)**	**+** *N = 4–5; males only*	**No nonexposed groups indicated (internal comparisons);** *acute exposure;* all animals	**Blocker experiments may be influenced by prior exposure to formaldehyde**	**+** *Effects of blocker experiments without*	**Not informative** [High formalin levels; etc.]

	Experimental Feature Categories ***Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated***					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for hazard ID**
			received anesthesia, surgery, and anticoagulants (no recovery before exposure)	Note: endpoint not considered adverse	*prior HCHO exposure NR*	
(Yu and Blessing, 1997)	**Formalin (likely high concentration- not quantified: methanol may drive responses); HCHO concentrations NR**	**+** *N = 5–16; males only*	**No nonexposed groups indicated (internal comparisons);** *acute exposure;* all animals received surgery, anesthesia, and catheterization 1 wk prior to exposure	**++** Note: Endpoint not adverse	**+** Data were pooled across groups for some measures Note: all comparisons to preexposure measures	**Not informative** [Formalin levels NR; etc.]
(Yu and Blessing, 1999)	**Test article not defined (assumed to be formalin); levels not quantified (likely high: methanol may drive responses)**	**+** *N = 4; males only*	**No nonexposed groups indicated (internal comparisons); other alerting & noxious stimuli administered pre-HCHO; 2 surgeries- only 1 d recovery after cannulation before exposure***; acute exposure*	**++** Note: Endpoint not adverse	**+** Justification for selection of resting periods used for comparison unclear; *data qualitative only*	**Not informative** [Test article assumed to be formalin; exposure levels NR; etc.]

NR = not reported; N/A = not applicable;

* Three studies examined an endpoint that is not adverse and has no MOA relevance. These are briefly mentioned in the assessment, as they inform the irritant/odorant threshold of rodents, but these studies were not used to characterize the potential neurotoxicity hazard.

** Five animal studies sufficient for hazard characterization were not categorized using confidence ratings, and they are not included in the exposure-response array, as they represent cursory observations with none or minimal data reporting; however, these studies were used to help describe the potential neurotoxicity hazard.

[a] See the draft Methanol Toxicological Review (http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=233771), which proposes an RfC of ≈2 mg/m^3. Assuming methanol is present in the breathing zone somewhere in the range of 1/10–1/3 the levels of formaldehyde when stabilized formalin solutions are used as the test article (determination of the exact ratio of exposure is not currently available), exposures > 10 mg/m^3 are assumed to have at least some methanol-driven effects.

[b] Kerns is a report of a GLP study by CIIT (Battelle, 1982), which was not identified in the literature search [Note: use of GLP or guideline study protocols is provided to identify the most stringent studies, but did not factor into the confidence ratings or sufficiency evaluations for this particular database].

[c] Communication with the study author detailed that male rats (2 per litter from 3 separate dams per dose group) were used in the Sarsilmaz et al. (2007) study. A review from this same laboratory (Songur et al., 2010) indicated that the stereological studies of the hippocampus were conducted to confirm previous observations (Songur et al., 2003); thus, the separate reports of stereological changes in the CA and DG regions of the hippocampus (Sarsilimaz et al. (2007) and Aslan et al. (2006), respectively) are assumed to represent the same cohort of animals (note: it is possible that these two stereological studies report effects on a subset of the same animals used in the Songur et al. (2003) study, but this inference is less clear and is not assumed).

d Note: although pup body weight changes would be of concern as potential confounders for behavioral analyses, endpoints such as neuropathology and brain weight are unlikely to be secondary to these changes: at least for brain weight, the current literature does not support a consistent causal relationship. In Songur et al. (2003), body weight decreases were ≈10% and 20% at 30 d (low and high formaldehyde concentrations, respectively) & ≈10% at 60 d (high concentration only).

[e] Because data for exposure groups other than 6.15 mg/m^3 were not reported by Boja et al. (1985), the higher exposure groups were not included in the study quality analysis or the Toxicological Review hazard ID synthesis.

Studies Specific to Mechanistic Considerations Only

Studies examining mechanistic events related to nervous system effects were systematically evaluated in order to inform biological plausibility. The evaluations included herein only encompass animal studies reporting mechanistic results following in vivo inhalation exposures (including exposures to animals under anesthesia or after surgery). Noninhalation (e.g., oral, i.p.) animal exposures are expected to involve a different distribution of formaldehyde to systemic sites such as the nervous system, as compared to inhalation exposure, and thus are likely to involve mechanisms unrelated to those observed following inhalation. Similarly, in vitro examinations were also not considered to be informative enough to warrant study quality evaluations, as appreciable amounts of formaldehyde are unlikely to reach the target cells in the nervous system following inhalation exposure. Notably, the aqueous formaldehyde solutions used in both in vitro and noninhalation in vivo studies typically contained methanol as a stabilizer, introducing additional uncertainties.

Although parallel criteria to those used to evaluate studies describing potential neurotoxicity hazards (see above) were used to judge the mechanistic studies, the stringency of some criteria were adapted to accommodate this type of information and additional leniency was applied for certain parameters (e.g., acute exposure was not considered a limitation). Studies are organized alphabetically.

Table A-87. Evaluation of studies pertaining to mechanistic events associated with nervous system effects

	Experimental Feature Categories *Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated*					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for MOA**
Criteria relevant to evaluating the experimental details within each experimental feature category[a]	Exposure quality evaluations (see B.4.1.2) are summarized below; "++": robust; "+": adequate; and shaded box: poor; relevance of the tested exposure levels is discussed in the hazard synthesis	The species, sex, strain, and age are appropriate for the endpoint(s); sample size provides reasonable power to assess the endpoint(s); overt systemic toxicity is absent or not expected, or it is accounted for; selection bias minimized	A study focus was nervous system effects; the exposure regimen is informative for the tested endpoint(s); acute exposure not necessarily a limitation; manipulations other than formaldehyde exposure are adequately controlled	Endpoint evaluates a mechanism relevant to humans[b]; protocols are complete, sensitive, discriminating, & biologically sound; experimenter bias minimized	Statistical methods, group comparisons, and data presentation (including variability) are complete, appropriate, and discerning; selective reporting bias avoided	[Main limitations] Expert judgement based on conclusions from evaluation of the 5 experimental feature categories
(Ahmed et al., 2007)	++	+ *N = 4–5; females only*	**Lack of OVA-free controls: inability to separate effects of OVA & formaldehyde; possible altered distribution/effectiveness of aerosolized OVA given after formaldehyde;** Note: 12 wk exposure; single exposure level	++	++	**Medium** [Control group deficiencies]
(Bian et al., 2012)	**Formalin (high concentration: methanol may drive effects)**	***N* = 3/endpoint/timepoint (males); mild toxicity: decreased food intake (effect not quantified)**	**Controls not air-exposed in exposure chamber; all groups had anesthesia & antibiotic injections** Note: exposure 1 hr/d for 90 d; single exposure level	++	++	**Not informative** [High formalin levels; etc.]
(Boja et al., 1985)	+ *Analytical concentrations NR*	+ *N* = 8; *males only*; *data from experiments with N=1 (air-HCHO NE & DA levels) not included in the assessment*	+ *Timing of exposures (9–12 pm vs. 12–3 pm) may have varied across groups* Note: single exposure level; acute exposure: 3 hr/d for 1–2 d	+ *Molecular verification of regional "punches" not performed*	+ *Higher exposure groups data NR*; inability to evaluate findings for exposures indicated as tested but NR	**Medium** [Selective reporting; some methods detail NR]

	Experimental Feature Categories *Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated*					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for MOA**
(Bokina et al., 1976)	**Details of exposure were not provided**	**Details on test subjects were not provided**	**Details of study design were not provided** Note: continuous exposure for 45d	**Details of endpoint measures were not provided**	**No quantitative comparisons to controls were performed**	**Not informative** [Experimental details NR]
(Fujimaki et al., 2004b)	**+** *Analytical concentrations NR*	**+** *N = 5–6; females only;* unclear influence of splenic effects (e.g., decreased weight)	**+** For OVA groups: *unclear if prior formaldehyde exposure had nasal effects influencing inhaled OVA booster distribution/effects*; Note: 12 wk exposure	**+** *Methods for ELISA of plasma NR*: assumed to be same as BAL fluid ELISA	**++**	**Medium** [Control group deficiencies; some methods detail NR]
(Fujimaki et al., 2004a)	**+** Analytical concentrations NR	**+** *ELISA data: N=5; males only* **RT-PCR data: *N*=3; (considered major limitation)**	**+** for OVA groups: *unclear if prior formaldehyde exposure had nasal effects influencing inhaled OVA booster distribution/effects*; 12 wk exposure	**Methods for brain dissection & homogenization, as well as gel quantification NR;** ELISA and booster challenge methods NR	**++**	ELISA: **Medium** RT-PCR: **Low** [Control group deficiencies; small sample size; some methods detail NR]
(Gieroba et al., 1994)	**Formalin (likely high concentration- not quantified: methanol may drive response)**	***N*= 2 or 6**	**Unclear contribution of apnea & bradycardia; results may be specific to exposure combined with restraint & anesthesia; strong irritation induced**	**+** *Number of sections analyzed/animal NR*	**Immunostaining results were not quantified across groups; results are qualitative only;** TH^+ cell counts alone NR	**Not informative** [High formalin levels; etc.]
(Hayashi et al., 2004)	**++**	**+** *N = 5; females only*	**++** Exposures up to 12 wk	**+** *Possible mild sampling bias* (3 sections, but selection methods NR); blinding indicated	**++**	**High**

	Experimental Feature Categories *Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated*					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for MOA**
(Kimura et al., 2010)	**Formalin**	***N* = 5-6; males only; systemic toxicity not evaluated (HCHO tested up to ~55 mg/m³)**	**+** *Irritation-related effects probable, as tested near-simultaneous with exposures;* acute exposure; unclear if anesthesia/dye injection influenced sensory nerve responses	**+** *Blinding not indicated* for cell type counts	**++**	**Low** [Formalin; possible overt toxicity]
(Kulle and Cooper, 1975)	**+** Analytical concentrations NR	***N*=3; males only; no air-only controls**	**+** All animals underwent surgery prior to exposure (*no recovery prior to exposure*); *some exposures were complicated by amyl alcohol co-exposure*; acute exposure	**++** Note: unclear relevance of these surgical preparations to human nerve responses	**No quantitative comparisons to controls performed (extrapolated threshold only)**	**Low** [small sample size; comparison group deficiencies]
(Chonglei et al., 2012)	**Mixture (formalin, benzene, toluene and xylene)/static chamber**	**+** *N= 5 males*/group	**++** 2 hr/d exposure for short term (10 d)	**No description of hippocampal MDA and GSH protocols provided**	**++**	**Not informative** [Mixture exposure; etc.]
(Li et al., 2016)	**Formalin; static chambers**	+ *N* = 7 (inferred); *males only*	**++** 2 hr/d exposure for short term (7 d)	+ *Some sampling bias possible: 3 sections* Note: although not corrected for neuron number, location determined from atlas; slides were randomized and coded for blinded evaluation	**++**	**Low** [Formalin]
(Liao et al., 2010) **(translation)**	**Formalin/static chamber**	***N*=8: pooled sexes (*N*=4/sex); overt toxicity during**	**+** *No indication of correction for possible litter bias*;	**Potential sampling bias: *N*=5 fields (assumed to be per**	**+**	**Not informative** [Formalin; endpoint protocol deficiencies; overt toxicity]

	Experimental Feature Categories *Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated*					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for MOA**
		exposure (e.g., listlessness; up to ≈30% decreased body weight gain), most likely from poor exposure quality, as only 0.5mg/m^3 HCHO	Note: 2 hr/d for 28 d	**animal), but number of slides not indicated (DAB amplification used) & no correction made to account for the number of neurons visible/field**	*Data= combined sexes; CA3 cell number or viability measures NR*	
(Liu et al., 2009a)	**Formalin (high concentration: methanol may drive effects)/static chamber**	**+** *N = 5; males only*	**++** 28 d exposures	**++**	**++**	**Not informative** [High formalin levels; etc.]
(Liu et al., 2010)	**Formalin (high concentration: methanol may drive effects)/static chamber**	**+** *N=5; males only;* treatment groups determined by preexposure probe trial performance, but method for matching groups NR	**++** 28 d exposures	**Methods for quantification of western blots NR**	**++**	**Not informative** [High formalin levels; etc.]
(LICM, 2008)	**Unspecified wood**	**+** Sample sizes for MOA-related endpoints were NR, but assumed to be *N=5; males only*	**++** 7 d exposures	**Regional brain dissections were nonspecific & methods incompletely described; RT-PCR analyses were semi-quantitative only**	**++**	**Low** [Possible mixture exposure; endpoint protocol description insufficient]
(Matsuoka et al., 2010)	**Formalin**	**+** *N*=7–9; *males only*	**+** *Did not appear that controls were air-exposed in chambers* ("noninhalation controls"); acute exposure	**Methods for brain dissection/regions analyzed NR**; assumed brain region-specific	**+** High variability in measures, *possibly due to lack of regional specificity*	**Low** [Formalin; endpoint protocol description insufficient]

	Experimental Feature Categories *Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated*					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for MOA**
				analyses were not conducted		
(Mei et al., 2016)	**Formalin**	**+** *N* = 8; *males only*	**+** *No comparisons to chamber or air exposure alone;* 8 hr/d for 7 consecutive d	**No blinding for biochemical measures; no regional specificity (homogenates)**	**++**	**Low** [formalin; some endpoint protocol limitations]
(Nalivaiko et al., 2003)	**Unregulated exposure without reporting of levels; no chamber** Note: paraformaldehyde	**+** *N* = 6–13; *males only*	**+** *No nonexposed groups indicated* (internal comparisons); all animals were implanted with electrodes, but *duration prior to testing not provided*; acute exposure	**+** *ECG implantation procedures NR*	**++**	**Not informative** [Exposure levels NR and unregulated; etc.]
(Ozen et al., 2003a)	**+** *Analytical concentrations NR*	**Unclear contribution of unexplained overt toxicity (robust effects on body weight); males only;** *N* = 7	**++** 4 wk or 13 wk exposures	**Methods for analyses of brain tissue were not clearly described, even in cited reference**	**++**	**Not informative** [Overt toxicity; endpoint protocol description insufficient]
(Sari et al., 2004)	**++**	**+** *N=5*/endpoint; *females only*	**++** 12 wk exposure	**Cell counts were not reported as observer blinded, but were from serial sections; RT-PCR analyses were semi-quantitative only**	**++**	**Medium** [possible experimenter bias- no blinding]
(Sari et al., 2005)	**++**	**+** *N = 5; females only*	**Nasal instillation of toluene may affect formaldehyde distribution**	**Cell counts were not reported as observer blinded, but were from serial sections**	**Data for exposures without toluene NR** Note: 2004 paper data cited was not considered	**Not informative** [Data on formaldehyde exposure alone NR; etc.]
(Songur et al., 2003)	**+** *Analytical concentrations NR*	***N* = 6 (assumed 3 litters); mild toxicity (body weight &**	**+** *Litter assignments NR; unclear if litter bias*	**Potential sampling bias: details on blinding,**	**+**	**Low**

	Experimental Feature Categories *Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated*					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for MOA**
		food/water intake changes): HSP activation may be indirectly related to health/nutrition	*corrected;* 30d of exposure	**slides/animal, etc. not provided; nonblinded intensity ratings subject to observer bias**	*No statistical comparisons for HSP staining*	[small sample size; possible litter and/or sampling bias]
(Songur et al., 2008)	++	**Dam health during lactation & pup health not presented; sex and litters/group unknown (likely males & 3 litters);** body weights were indicated as measured, but NR; *N* = 7 pups	+ *Unclear if litter bias corrected (& not indicated as randomized)*; dams exposed from PND1-14; 30 d of exposure	++	++	**Medium** [Small sample size; possibly litter effects]
(Sorg et al., 2001a)	++	+ *N = 6–10; males only*	+ *Possible difference in harvest day* (20 vs 21) across groups may contribute to high variability noted in results; exposures ≤4 wk	+ Volume of trunk blood/animal and some other details (e.g., serum isolation) NR Note: chamber exposure itself (tested) had a large influence, so critical to rapidly remove rats after exposure (as indicated)	++ Note: sensitive endpoint, so high level of variability is as expected	**High**
(Sorg et al., 2002)	**Formalin (likely high concentration; not quantified: methanol may drive response)**	+ *N* = 6–12	**Formalin used as an aversive stimulus- results more specific to cocaine;** acute exposure to concentrated vapors	**Tests involve odor detection & irritation-specific responses could be confounding results**	+ *Specific effects of formaldehyde alone not tested or NR*	**Not informative** [Formalin (assumed high level) levels NR]
(Tani et al., 1986)	**Formalin (high concentration:**	+ *N = 4–5; males only*	+ *No nonexposed groups indicated (internal*	+ *Blocker experiments may be influenced by*	++	**Not informative** [High formalin levels]

	Experimental Feature Categories *Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated*					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for MOA**
	methanol may drive responses)		*comparisons); animals received anesthesia, surgery, and drugs with no recovery before exposure*; acute exposure	*prior exposure to formaldehyde* (not tested)		
(Tsukahara et al., 2006)	++	+ *Females only; Western Blot data: N≥ 6;* **Caspase data: *N*=3; (considered major limitation)**	+ For OVA groups: *unclear if prior formaldehyde exposure had nasal effects influencing inhaled OVA booster distribution/effects*; 60d exposure	++ (for Western Blot data) **Caspase data: likely sampling bias: number of slides/animal & neurons visible/field NR; counts were not reported as observer blinded**	++	Western blot: **High** Caspase: **Low** [Caspase data: small sample size; likely sampling bias]
(Wang et al., 2014a)	**Mixture (formalin, benzene, toluene and xylene)/static chamber**	+ N = 6–12; males only Note: no changes in body weight were observed	++ 2 hr/d exposure for subchronic (90 d); tested 1 d postexposure	**No description of grip strength protocol provided**	++	**Not informative** [Mixture exposure; endpoint protocol NR]
(Yu and Blessing, 1997)	**Formalin (likely high concentration; not quantified: methanol may drive responses)**	+ *N = 5–16; males only*	**Animals received surgery, anesthesia, & catheterization 1 wk prior to exposures; no nonexposed groups indicated (internal comparisons)**; acute exposure	++	+ *Data was pooled across groups for some measures* Note: all comparisons to preexposure measures	**Not informative** [Formalin (assumed high level) levels NR; etc.]
(Yu and Blessing, 1999)	**Test article not defined (assumed to be formalin); levels not quantified (likely high: methanol may drive responses)**	+ *N = 4; males only*	**No nonexposed groups indicated (internal comparisons); other alerting & noxious stimuli administered pre-HCHO; 2 surgeries; only 1 d**	++	+ Justification for selection of resting periods used for comparison unclear; *data qualitative only*	**Not informative** [Unknown test article (assumed to be formalin) levels NR (assumed high level); etc.]

	Experimental Feature Categories *Study detail(s) supporting a major (bolded) or minor (italicized) experimental feature limitation is indicated*					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation**	**Data considerations & statistical analyses**	**Overall confidence rating regarding the use for MOA**
			recovery after cannulation before exposure; acute exposure			
(Zitting et al., 1982)	**Test article results in co-exposures to formic acid, acrolein, & possibly other chemicals**	**+** *N = 4–5; males only*	**Formaldehyde levels >> 100 mg/m^3 are overtly toxic (rats gasped for air for hours after exposure);** 6 hr or 3 d exposure	**+** *Evaluations are not brain-region-specific*	**+** Details on statistics NR (e.g., "Student's *t* test")	**Not informative** [Unknown test article (assumed to be formalin) at high level; overt toxicity]

[a] Mode-of-action study quality evaluations were conducted in a similar fashion as those described above for hazard identification, with minor adjustments to the types of experimental details considered for meeting sufficiency criteria (e.g., adversity of the endpoint was not considered).

[b] A mechanism or mode of action is considered relevant to humans unless there is convincing evidence to the contrary.

A.5.8. Developmental and Reproductive Toxicity

Literature Search

A systematic evaluation of the literature database on studies examining the potential for noncancer developmental and/or reproductive effects in humans or animals in relation to formaldehyde exposure was initially conducted in October 2012, with yearly updates to September 2016 (see A.5.1). A systematic evidence map identified literature published from 2017 to 2021 (see Appendix F). The search strings used in specific databases are shown in Table A-88**.** Additional search strategies included:

- Review of reference lists in the articles identified through the full screening process.
- Review of reference lists in the 2010 draft Toxicological Review for Formaldehyde (U.S. EPA, 2010), the ATSDR toxicological profile of formaldehyde (ATSDR, 1999), and the NTP report on carcinogens background document for formaldehyde (NTP, 2010).
- Review of references in 41 review articles relating to formaldehyde and reproductive or developmental effects, published in English, identified in the initial database search. References were retrieved through Web of Science and added to the database.

This review focused on reproductive effects in women and men, fetal loss (e.g., spontaneous abortion), and birth outcomes. Effects in animals included alterations in pre- and postnatal development (survival, growth, structural alterations) and in the integrity of the male and female reproductive system (cells/tissues/organs, outcomes, and function). Inclusion and exclusion criteria used in the screening step are described in Table A-89 and Table A-90, respectively, for human and animal studies.

After manual review and removal of duplication citations, the 9,854 articles identified from database and additional searches were initially screened within an EndNote library for relevance; title was considered first, and then abstract in this process. Full text review was conducted on 261 identified articles. The search and screening strategy, including exclusion categories applied and the number of articles excluded within each exclusion category, is summarized in Figure A-36. Based on this process, 55 studies were identified and evaluated for consideration in the Toxicological Review.

Table A-88. Summary of search terms for developmental or reproductive toxicity

Database, search date	Terms
PubMed No date restriction	(formaldehyde [majr] OR paraformaldehyde OR formalin) AND ("reproductive toxicity" OR "reproductive toxicology" OR reproductive OR "developmental toxicity" OR "developmental toxicology" OR development OR developmental OR ontogen* OR "embryo toxicity" OR embryo OR embryon* OR embryog* OR embryot* OR "fetal loss" OR fetal OR fetus OR fetuses OR fetotoxi* OR miscarriage or miscarry OR "spontaneous abortion" OR "preimplantation loss" OR preimplantation OR "postimplantation loss" OR postimplantation OR implantation OR conception OR resorption OR fertility OR fertile OR infertility OR infertile OR pregnancy OR gestation OR neonatal OR neonate OR prenatal OR postnatal OR "menstrual cycle" OR "premature birth" OR "preterm birth" OR "low birth weight" OR "in utero" OR "fetal body weight" OR "fetal weight" OR pup OR "pup body weight" OR "pup weight" OR ovary OR ovaries OR ovu* OR sperm OR gamete OR "germ cells" OR "Sertoli cells" OR testes OR testis OR testic* OR uterus OR uteri* OR epididy* OR prostate OR "seminal vesicles" OR semen OR testosterone OR "luteinizing hormone" OR LH OR "follicle stimulating hormone" OR FSH OR estrogen OR estradiol OR "time to pregnancy" OR "time-to-pregnancy" OR TTP OR fecund*)
	NOT (fixative OR "formaldehyde fixation" OR "paraformaldehyde fixation" OR "formalin fixation" OR "formaldehyde fixed" or "paraformaldehyde fixed" OR "formalin fixed" OR "formaldehyde-fixed" or "paraformaldehyde-fixed" OR "formalin-fixed" OR formocresol OR dental OR dentistry OR immunogen OR vaccine OR vaccination OR metabolite) [Note: for quality control, ≈1% (75) of the 7,589 excluded article titles were scanned in PubMed: 2 potentially relevant government reports were found and 4 duplicates were excluded, resulting in 2,810 in the final database.
Web of Science No date restriction Lemmatization "off"	SU=(Toxicology OR "Pharmacology &Pharmacy" OR "Public, Environmental & Occupational Health" OR "Cell Biology" OR "Reproductive Biology" OR "Biochemistry & Molecular Biology" OR Pathology OR "Obstetrics & Gynecology" OR "Environmental Sciences" OR "Anatomy & Morphology" OR Andrology OR "Veterinary Sciences" OR Physiology OR "Developmental Biology" OR "Research & Experimental Medicine" OR "Life Sciences Biomedicine Other Topics" OR "Veterinary Sciences") AND TS=(formaldehyde OR paraformaldehyde OR formalin) AND TS=(formaldehyde OR paraformaldehyde OR formalin) AND TS=(formaldehyde OR paraformaldehyde OR formalin) AND TS=("reproductive toxicity" OR "reproductive toxicology" OR reproductive OR "developmental toxicity" OR "developmental toxicology" OR development OR developmental OR ontogen* OR "embryo toxicity" OR embryo OR embryon* OR embryog* OR embryot* OR "fetal loss" OR fetal OR fetus OR fetuses OR fetotoxi* OR miscarriage or miscarry OR "spontaneous abortion" OR "preimplantation loss" OR preimplantation OR "postimplantation loss" OR postimplantation OR implantation OR conception OR resorption OR fertility OR fertile OR infertility OR infertile OR pregnancy OR gestation OR neonatal OR neonate OR prenatal OR postnatal OR "menstrual cycle" OR "premature birth" OR "preterm birth" OR "low birth weight" OR "in utero" OR "fetal body weight" OR "fetal weight" OR pup OR "pup body weight" OR "pup weight" OR ovary OR ovaries OR ovu* OR sperm OR gamete OR "germ cells" OR "Sertoli cells" OR testes OR testis OR testic* OR uterus OR uteri* OR epididy* OR prostate OR "seminal vesicles" OR semen OR testosterone OR "luteinizing hormone" OR LH OR "follicle stimulating hormone" OR FSH OR estrogen OR estradiol OR "time to pregnancy" OR "time-to-pregnancy" OR TTP OR fecund*)
	NOT (fixative OR "formaldehyde fixation" OR "paraformaldehyde fixation" OR "formalin fixation" OR "formaldehyde fixed" or "paraformaldehyde fixed" OR "formalin fixed" OR "formaldehyde-fixed" or "paraformaldehyde-fixed" OR "formalin-fixed" OR formocresol OR dental OR dentistry OR immunogen OR vaccine OR vaccination OR metabolite)

Database, search date	Terms
	[Note: for quality control, ≈2% (40) of the 2,309 excluded article titles were scanned in Web of Science: none were relevant].
ToxNet (Toxline and DART) No date restriction	(formaldehyde OR paraformaldehyde OR formalin) AND ("reproductive toxicity" OR "reproductive toxicology" OR reproductive OR "developmental toxicity" OR "developmental toxicology" OR developmental) (including synonyms and CAS numbers, but excluding PubMed records); 525 identified; 11 discarded upon importation into EndNote because they were duplicates

Table A-89. Inclusion and exclusion criteria for studies of reproductive and developmental effects in humans

	Included	Excluded
Population	**Human**	**Animals**
Exposure	• Indoor exposure via inhalation to formaldehyde • Measurements of formaldehyde concentration in air • Formaldehyde-specific assessments in exposed occupations (wood workers, nurses, pathologists, cosmetologists)	• Not formaldehyde • Outdoor formaldehyde exposure • Mixtures or industry/job title analyses • Not inhalation
Comparison		• Case reports
Outcome	• Reproductive toxicity (sperm measures) • Time-to-pregnancy (fecundity) • Spontaneous abortion • Pregnancy • Birth outcomes	• Exposure studies/no outcomes evaluated • Other health outcomes not related to reproduction or development
Other		• Reviews, reports, meeting abstract, methodology paper, laboratory techniques using formalin, mechanistic studies, foreign language

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Table A-90. Inclusion and exclusion criteria for studies of reproductive and developmental effects in animals

	Included	Excluded
Population	• Experimental animals • Nonmammalian test species or test paradigms that are relevant for evaluation or developmental or reproductive hazard	• Humans • Irrelevant species or test paradigms
Exposure	• Inhalation route, formaldehyde	• Not formaldehyde • Noninhalation routes of exposure • Mixture studies • Ecological studies
Comparison	• Inclusion of a comparison group (e.g., pre- or postexposure, no exposure, vehicle exposure, lower formaldehyde exposure level)	• No comparison group
Outcome	• Pre- and postnatal offspring biomarkers of: o Survival (e.g., resorptions, death) o Growth (e.g., body weight) o Structural anomalies (e.g., external, skeletal, or soft tissue malformations or variations) o Functional deficits • Adult biomarkers of reproductive toxicity, including: o Gonadotropic hormone measures o Reproductive organ weight o Reproductive organ macro- and microscopic pathology o Sperm measures (count, motility, morphology) o Reproductive function (e.g., mating, fertility, parturition, gestation, lactation) o Mechanistic data relevant to developmental or reproductive outcomes	• No health outcomes evaluated • Health outcomes not related to developmental or reproductive toxicity • Mechanistic data irrelevant to developmental or reproductive outcomes
Other	• Original primary research	• Not original primary research, e.g., reviews, reports, commentaries, meeting abstracts, policy papers • Duplicates, or untranslated foreign language studies (judged to be irrelevant or unlikely to have a significant impact, based on review of title, abstract, and/or tables/figures) • Methodology papers, or studies describing laboratory techniques using formaldehyde

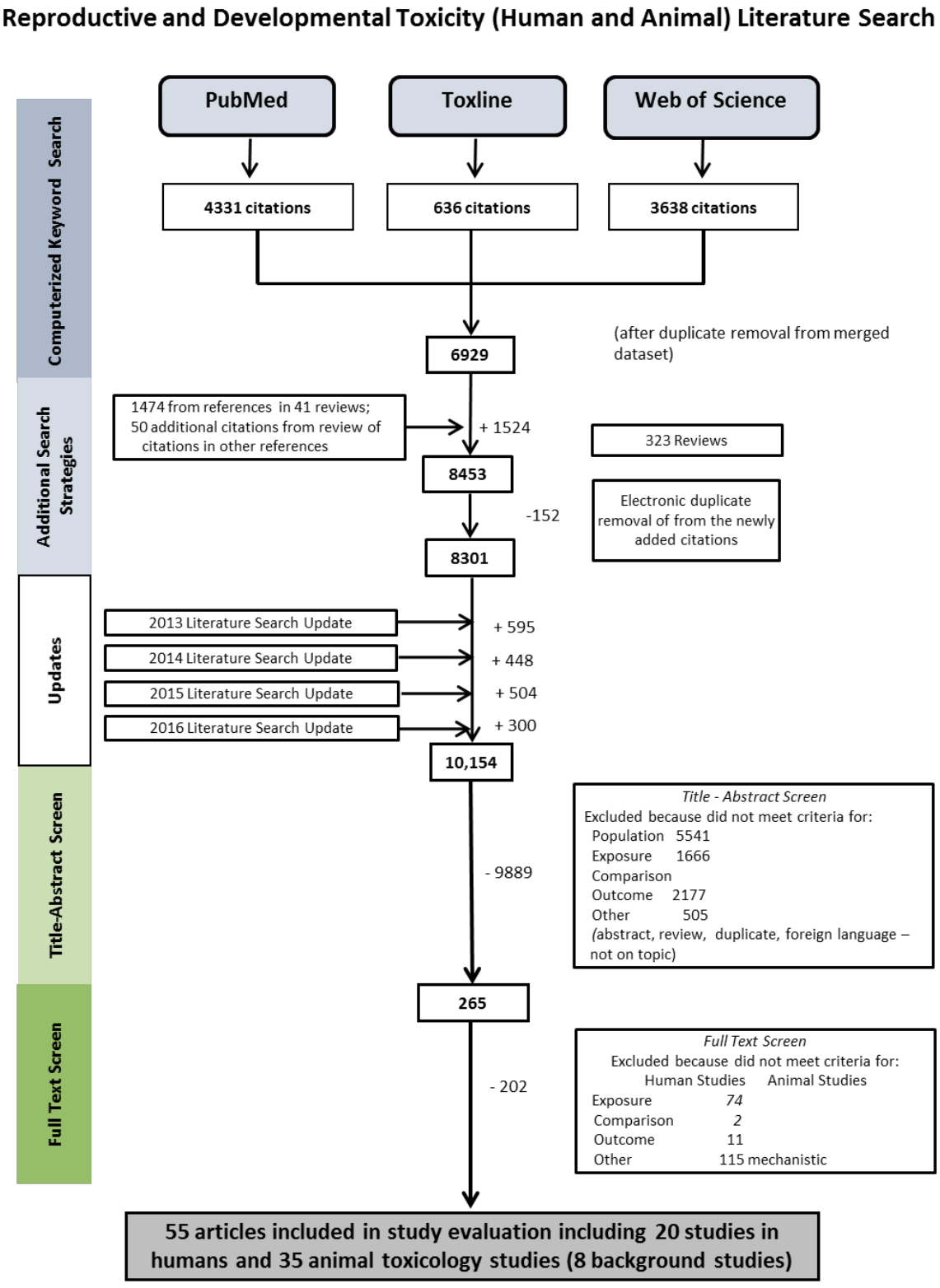


Figure A-36. Literature search documentation for sources of primary data pertaining to formaldehyde exposure and developmental and reproductive toxicity.

Study Evaluations

Human Studies

Participant Selection

Occupational studies of spontaneous abortion may be influenced by selection bias if participants are recruited from current employees. This is because women with a history of spontaneous abortion are more prevalent in the working population (Axelsson, 1984). Time-to-pregnancy also may be increased among current workers because early spontaneous abortion contributes to this effect (Slama et al., 2014; Baird et al., 1986). Four of the reviewed studies reduced the potential for selection bias by recruiting from union rosters, registers of licensed practitioners, or graduate school enrollment lists (Taskinen et al., 1999; Steele and Wilkins, 1996; John et al., 1994; Taskinen et al., 1994). Another case-control study identified spontaneous abortion events from a nationwide hospital discharge register (Lindbohm et al., 1991). Thus, selection into the study was not conditional on being currently employed in the industry at the time of the study. Regardless of the method used to identify the study population, most of the studies used an appropriate comparison—other employed individuals. Generally, participation rates reported by study authors were above 70%; thus, participants likely were representative of the population under study.

Another potential bias may result from which pregnancy (first, pregnancy during defined time period, most recent) is selected as the index pregnancy in studies of spontaneous abortion. Studies that focus on the most recent pregnancy may be less sensitive due to time-lapse bias. The time between a pregnancy ending in spontaneous abortion and a subsequent pregnancy ending in a live birth is often shorter than two pregnancies, both ending in live births. This can result in a bias toward identifying live births as the most recent pregnancy (Wilcox, 2010).

Outcome ascertainment

The validity of retrospectively collected self-completed questionnaire data on time-to-pregnancy has been evaluated by some authors and was found to closely reproduce the distributions of TTP in the group using a different data source (e.g., data collected during annual follow-up of a family planning cohort) (Joffe et al., 1995). This finding suggests that data from the questionnaires can be used to differentiate differences between groups. The comparability of the distributions based on the two data sources persisted even among individuals for whom the duration of recall was greater than 14 years. In addition, subfertility, defined as a TTP greater than 12 months using the questionnaires, was identified with high sensitivity (79.9%) and specificity (94.9%) (Joffe et al., 1993). However, individuals recalled the number of months before conception with greater error, and these errors increased as the duration of time-to-pregnancy increased. Longer TTP was both over- and under-estimated (Cooney et al., 2009; Joffe et al., 1995). Therefore, while individual estimates of TTP may be less precise, the comparison of group means with respect to levels of formaldehyde exposure is likely to be informative. The studies of TTP and

formaldehyde exposure collected information about these variables in the same questionnaire; thus, making it difficult to exclude the possibility that recall of TTP may have been differential with respect to exposure status.

Validity studies indicate that recall of previous spontaneous abortions is relatively complete, particularly for losses that occurred after the 8th week of gestation (> 80% of recorded spontaneous abortions were recalled) (Wilcox and Horney, 1984). Completeness varies by occupation; completeness of recall among nurses was better than that among industrial workers (Lindbohm and Hemminki, 1988; Axelsson and Rylander, 1982). Although elapsed time since the event occurred may also influence the completeness of recall, this also varied by occupation in a similar way (not important among nurses) and was not important within the first 10 years after the event (Lindbohm and Hemminki, 1988; Wilcox and Horney, 1984). It is difficult to evaluate the validity of self-reports of spontaneous abortion occurring during the 1st trimester using medical records because these early events often are not recognized or do not require medical intervention; medical records may not necessarily be an accurate reference (Slama et al., 2014; Lindbohm and Hemminki, 1988).

The degree to which the ability to recall a spontaneous abortion or a decision to participate in the study may be associated with exposure status will affect the potential for bias with either overestimation or underestimation of effect estimates (Slama et al., 2014). Several of the studies identified both cases and referents from the same occupational database or source population, thus reducing the likelihood that recall was associated with formaldehyde exposure (Taskinen et al., 1999; Steele and Wilkins, 1996; John et al., 1994; Taskinen et al., 1994). However, selection bias has been documented in studies of spontaneous abortion within an occupational group. A study of exposure to anesthetics among current and previous health personnel at a hospital in Sweden reported a higher response rate among exposed cases (Axelsson and Rylander, 1982). While the rate of response to the mailed questionnaire was relatively high and comparable between the exposed (85%) and unexposed (84%) female hospital personnel, an additional 20 spontaneous abortions were found in hospital records for unexposed nonrespondents, whereas no additional cases were found among exposed nonrespondents. It is difficult to predict the magnitude of the impact of this potential selection bias on the findings of the reviewed studies, although it may vary depending on the occupation.

Evaluation of Possible Confounding

Variables associated with time-to-pregnancy include age, gravidity (any previous pregnancies), educational level, use of oral contraceptives, frequency of intercourse, recent pregnancy or breastfeeding, specific medical conditions, cigarette smoking, alcohol consumption, and radiation exposure (Baird, 1988; Baird et al., 1986; Baird and Wilcox, 1985). These individual characteristics are possible confounders of the relation between formaldehyde exposure and time-to-pregnancy if they are associated with formaldehyde exposure in the study population. Spontaneous abortions during the first trimester most commonly result from chromosomal

abnormalities, and risk factors include maternal and paternal age. Other factors associated with increased risk include previous pregnancy loss, cigarette smoking, alcohol consumption, and maternal health conditions (Wilcox, 2010, p. 153-157, p. 153-157). Almost all of the studies addressed these potential confounding factors through adjusted analyses or by matching on characteristics associated with spontaneous abortion risk. Adjusting for previous pregnancy loss or gravidity can be problematic and potentially result in biased effect estimates because past pregnancy history also may be related to exposure in ways that are part of the causal pathway. Therefore, adjustment for these parameters was considered a limitation.

Exposure Assessment

A variety of different approaches to the assessment of exposure were used in this set of studies. These ranged from more specific, robust measures such as estimates of time-weighted average concentrations (based on job-specific formaldehyde measurements and the proportion of time spent at the job reported by participants) (Wang et al., 2012; Taskinen et al., 1999; Seitz and Baron, 1990b) to measures subject to greater misclassification error, such as the self-reported use of specific products or chemicals, or assignment to exposures by supervisors. In the absence of formaldehyde measurements, studies assigned exposure based on self-report (Steele and Wilkins, 1996; John et al., 1994; Saurel-Cubizolles et al., 1994; Taskinen et al., 1994; Axelsson et al., 1984), an informed source (Hemminki et al., 1985; Hemminki et al., 1982) or occupation/industry codes from census data combined with expert knowledge of industry wide concentrations (Lindbohm et al., 1991). Studies that used an open-ended question about what chemical exposures a participant experienced were determined to be not informative and were excluded. The assignment to exposure categories by third parties (supervisors of the participants or industrial hygienists) likely resulted in an exposed group with large variation in exposure intensity and frequency with a reduction in sensitivity. Exposure misclassification and the classification of individuals with probable low or infrequent exposure as exposed was a major limitation in these and other studies designated as low confidence (Zhu et al., 2006, 2005; Lindbohm et al., 1991; Hemminki et al., 1985; Hemminki et al., 1982).

Exposure assignments based on responses to questionnaires are likely to be affected by the ability to recall exposures, resulting in misclassification. However, unless responses were influenced by the respondent's pregnancy outcome, the misclassification would more often result in an attenuation of the risk estimates. A study of women who worked in laboratories at a Swedish university provides some evidence that differential recall bias may be an important issue. Women who reported miscarriages that could not be verified in a national birth register, also reported a higher rate of exposure to solvents (Axelsson and Rylander, 1982). However, a few validity studies of questionnaire responses about exposure among women with adverse reproductive and pregnancy outcomes did not find evidence for differential recall bias. An investigation of the repeatability of reported exposures among women who experienced a miscarriage did not find an increase in reported occupational and residential exposures after the event (Farrow et al., 1996).

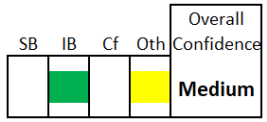
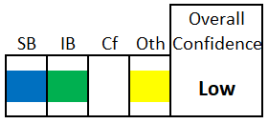
Other studies of questionnaire validity reported that sensitivity and specificity of responses to specific questions about chemical exposure were similar between individuals reporting a history of subfertility or adverse pregnancy outcomes, and individuals in the comparison groups (Joffe et al., 1993; Ahlborg, 1990). Notably, specificity was high for questions about specific chemicals, indicating that false positives for exposure were less likely. Further, other studies have found that under-reporting rather than over-reporting of exposures is more common (Joffe et al., 1993; Ahlborg, 1990; Hemminki et al., 1985). Therefore, while differential reporting of exposure by outcome status was evaluated for the studies of formaldehyde, it was not assumed to have occurred.

The criteria that were important in the evaluation of the studies for these endpoints are included in Table A-91 below. Information from the published studies pertinent to each of the evaluation categories was evaluated and conclusions are documented in the table that follows (see Table A-92). Studies are arranged alphabetically within each table.

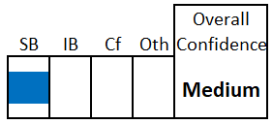
Table A-91. Criteria for categorizing study confidence in epidemiology studies of reproductive and developmental effects

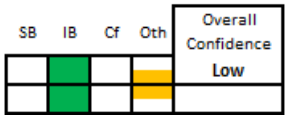
Study Confidence	Exposure	Study Design and Analysis
High	**Work settings**: Ability to differentiate between exposed and unexposed, or between low and high exposure. Exposure assessment specific to formaldehyde exposures and based on concentration data; includes assessment of intensity and frequency. Exposures characterized for etiologically relevant time window (e.g., period prior to or during pregnancy attempt for time-to-pregnancy or first trimester for spontaneous abortion).	Pregnancy outcomes compared between employed exposed and employed referent groups. Spontaneous abortion defined. Analytic approach evaluating dose-response relationship using analytic procedures that are suitable for the type of data, and quantitative results provided. Confounding considered and addressed in design or analysis; co-exposures (risk factors for endpoint) relevant to occupational setting addressed in analyses. Large sample size (n cases).
Medium	**Work settings**: Exposure assessment may not include formaldehyde concentration measurements, but other information used to differentiate between exposed and unexposed, or between low and high exposure levels. Incorporation of information on intensity and frequency. Referent group may be exposed to formaldehyde or to other exposures affecting reproductive or developmental outcomes (potentially leading to attenuated risk estimates).	One or a few limitations noted but otherwise study used a strong methodological and analytical design. While potential confounders may have been evaluated, co-exposures (risk factors for endpoint) relevant to occupational setting may not be.
Low	High likelihood of exposure misclassification and no information on frequency or intensity of exposure; imprecise assignment of exposure period to relevant time window for endpoint under study.	Evidence of confounding by other co-exposures in workplace and only single pollutant analyses presented; may be small number of exposed cases; not all important potential confounders addressed.
Not Informative	Use of an open question regarding occupational exposures.	Insufficient reporting detail; insufficient number of exposed cases ascertained; important potential confounders not addressed (age, gravidity, smoking).

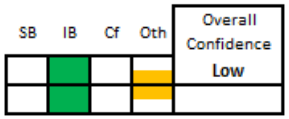
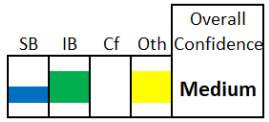
Table A-92. Evaluation of observational epidemiology studies of formaldehyde - reproductive and developmental outcomes

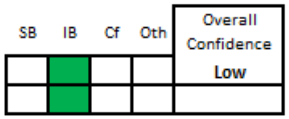
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Residential Studies							
Franklin et al. (2019) (Australia) Birth cohort	Pregnant women, all nonsmokers, recruited prior to 18 wks gestation. Recruited 373 women, 305 (81.7%) participated; 4 excluded because of smoking. Birth data available for 262 live births.	Air monitoring in homes at 34 wks gestation, 7-d sampling duration using validated passive samplers in bedroom and living room. LOD 2.4 µg/m^3; used LOD/2 for values < LOD. House average Median (range) 2.81 (LOD – 17.33) µg/m^3; 23.3% < LOD. Uncertainties in exposure distribution due to large % < LOD.	Gestational age, birth weight, birth length and head circumference from birth records.	Confounders were selected based on previous literature. Adjusted for maternal age, parity, maternal asthma, diabetes and blood pressure, season of birth. Distance from main road and ETS exposure were evaluated as potential confounders in models. Adjusted and unadjusted results presented.	Gestational age was normally distributed. Birth weight, birth length and head circumference were transformed to z-scores (accounting for sex and gestational age). General linear models.	N = 262	**Gestational age, birth weight, birth length, head circumference**  **Medium** Uncertainties in exposure distribution due to large % < LOD, small sample size, uncertain relationship between outcomes and window of exposure (3rd trimester)
Amiri and Turner-Henson (Southeastern United States) Cross sectional study	Pregnant women in 2nd trimester (convenience sample, n = 140) recruited from obstetrics and gynecology clinics with no history of chronic	Participants wore vapor monitor badges, 24-hr period, detection limit 0.003 ppm. Mean (SD) 0.04 (0.06) ppm = 0.049 (0.074) mg/m^3. This is a measure of total	Ultrasonographic biometry during 2nd trimester for head circumference, abdominal circumference, femur length, biparietal	Urine cotinine adjusted for urinary creatinine (spot sample, methods and timing of collection were not described). Models adjusted for maternal demographics,	Multiple linear regression for formaldehyde as dichotomous variable (cutoff at 0.03 ppm) adjusted for maternal age, fetal sex and	N = 88	Ultrasonographic biometry measurements  Low participation rate with no comparisons raises

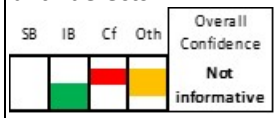
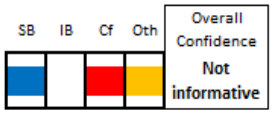
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	disease or high-risk pregnancy, 19–40 yrs old, Participation 63% (n = 88). No comparison of those who did and did not return the formaldehyde badges which raises a concern for selection bias.	exposure from indoors and ambient air.	diameter, estimated fetal weight, and ratio of abdominal circumference to femur length. Measurements in mm converted to percentiles using gestational age and the Hadlock formulas. Sensitivity and specificity for IUGR are 67% and 93% for BPD, 42% and 100% for HC, 94% and 100% for AC and 46% and 90% for AC/FL ratio. Hadlock formulas are based on a sample of White women in the US with uncertain accuracy for other races. Over 50% of the participants were not White.	obstetric history, and cotinine. Biometry measurements were not correlated with maternal age, education, marital status, yearly family income or employment status. No correlation with gravida, maternal smoking or pregnancy intervals. BPD was lower among whites compared to African-Americans or other category. BPD and FL varied by sex.	race. Mediation of tobacco smoke (urinary cotinine) on associations examined.		concern for selection bias. Small sample size with reduction in sensitivity. Reference population for BPD measure was not appropriate for >50% of participants.
Chang et al. (2017) (Birth	Pregnant women were selected from cohort (n = 383), originally	Personal formaldehyde measurements during mid- or late	Age-specific weights by gender using growth	Prenatal variables from questionnaire and medical records; postnatal via	Analyzed birth weight adjusted for maternal age, pre-	N = 360 singleton newborns	Birth weight; mean difference in weight at 6, 12, 24, and 36 mos

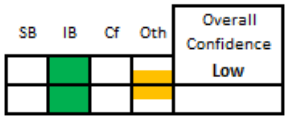
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
cohort) South Korea Mother and Childrens Environmental Health Study	recruited from hospital; information on demographics and housing characteristics via questionnaire. Infants followed at 6 (n=262), 12 (n=234), 24 (n=199), and 36 months (n=92).	pregnancy, 3 d. Categorized into two groups below and above the 75th percentile and also continuous with log transformation. Mean (SD) 0.082 (0.052) mg/m^3, geometric mean 0.067, 75th percentile 0.106 mg/m^3. Correlation between TVOCs and formaldehyde 0.22, p<0.01.	standard for Korean children.	questionnaire and interview. ETS slightly higher in low formaldehyde group but was not associated with weight.	pregnancy body mass index, education level, parity, gender, gestational age at birth and residential factors. Analyzed postnatal weight at each visit using multiple linear mixed models adjusted for gender, birth order, breastfeeding and education.		 Hospital-based cohort with potential selection bias, notable attrition over time
Occupational Studies							
(Axelsson et al., 1984) (case-cohort) laboratory work	University laboratory workers identified via payroll (born 1935 and after, worked in lab 1968–79); 95% response; birth register records compared for	Self-report (Y/N) during 1st trimester, open question; likely exposure misclassification, no information on intensity or frequency of exposure	Spontaneous abortion & birth defects; self-report & birth registry, 1968–1979. Spontaneous abortion verified using hospital records or via recall.	Miscarriage rate not associated with smoking before or during pregnancy (raises uncertainty about data quality); inverse association of solvent exposure with pregnancy number, age, and work shift	Unadjusted analyses for formaldehyde	Only 10 exposed pregnancies; potentially unstable risk estimates	**Spontaneous abortion Birth defects**  Open-ended question unreliable for exposure classification; uncertainty regarding data quality: miscarriage rate higher in nonresponders and not associated with smoking

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	respondents and nonrespondents.						
Ericson et al. (1984) (nested case control)	Controls (2 per case) selected from other infants in registry born in 1976 of laboratory worker; 50% of cases and 20% of controls responded about exposure	Lab work identified by occupational code in 1975 census; self-report on work during pregnancy & exposure to agents (open question); potential misclassification; no information on intensity or frequency of exposure	Perinatal deaths (< 7 d) & birth defects; National Birth Register, 1976	Controls selected randomly within same age (5-yr categories) and parity stratum as case. No information on smoking or other risk factors.	Unadjusted analyses for formaldehyde	3 exposed cases	**Perinatal deaths** **Birth defects**  Open-ended question unreliable for exposure classification; low response regarding exposure; very few exposed cases
Hemminki et al. (1982) (cohort) hospital staff	Recruited from nursing staff working in sterilizing units (exposed to sterilizing agents, x-rays, or anesthetic gases) or auxiliary units (referent) in all general hospitals; Response > 90% for both exposed and referent; recall likely not related to exposure	Exposure (Y/N) at beginning of pregnancy to specific agents assigned by supervising nurse, blind to case status, possible exposure misclassification, particularly for earlier years. No information on intensity and frequency.	Spontaneous abortion: self report on pregnancies, 1951–1981; questionnaire & hospital discharge register	Regression adjusted for several risk factors, and presented risk estimates for other sterilants (ethylene oxide, glutaraldehyde). Formaldehyde results not adjusted for other sterilants.	Binary logistic regression for exposure (yes/no) adjusted for age, parity, decade of pregnancy, smoking habits, alcohol, and coffee consumption	50 exposed pregnancies (6 spontaneous abortions); 1,100 unexposed pregnancies (121 spontaneous abortions)	**Spontaneous abortion**  Assumed sterilant use was same throughout period; no information on intensity and frequency of formaldehyde exposure (exposure misclassification—decreased sensitivity); no adjustment for other sterilants; adjustment for parity may introduce bias;

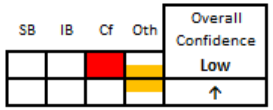
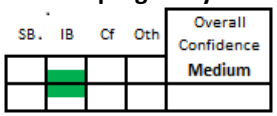
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Hemminki et al. (1985) (case control) nursing staff	Birth outcomes from national discharge register linked to national occupational register. Occupation identified for > 87% of exposed and referent. Selected hospital nurses.	Occupation during 1st trimester identified by head nurses at all general hospitals in Finland plus exposure (Y/N) to listed substances (used sterilizing agent or sterilized instruments; formaldehyde included in list); potential exposure misclassification; no information on intensity or frequency.	Spontaneous abortion & birth defects, 1973–1979; hospital discharge register linked to personnel register	Referent with healthy birth selected from same hospital as cases; matched on age; not adjusted for other risk factors or other workplace exposures	Conditional logistic regression. Unadjusted OR presented for FA; no statistical tests	6 exposed cases for spontaneous abortion 3 exposed cases for birth defects	**Spontaneous abortion and birth defects**  No information on intensity or frequency (exposure misclassification—decreased sensitivity); very small number of exposed cases
John et al. (1994) (case control) cosmetologists	Recruited from license registry (currently and formerly employed), 74% with eligible pregnancy, data obtained for 71.5% of cases, 74% live births; restricted analysis to full-time workers	Self-report; response to closed list (Y/N & frequency of use), no ambient measurements; relevant exposure period: 1st trimester; pregnancies while full-time cosmetologist	Spontaneous abortion, 1983 – 1988, most recent pregnancy (decreased sensitivity because of time-lapse bias). Self-report verified by positive pregnancy test or medical care	Regression adjusting for several risk factors plus other work exposures among full-time cosmetologists	Adjusted OR, 95% CI, unconditional logistic regression adjusting for previous pregnancy loss, mother's age at conception, & mother's cigarette smoking during 1st trimester	67 cases, 351 controls	**Spontaneous abortion**  Selection of most recent eligible pregnancy (decreased sensitivity); no ambient measurements; adjustment for previous pregnancy loss may introduce bias

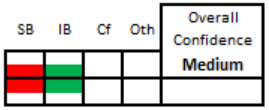
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	during 1st trimester.						
Lindbohm et al. (1991) (registry linkage) paternal occupation	Identified all pregnancies between 1/1/76–12/31/77 and 5/1/80 –4/30/82, excluded maternal age < 12 and > 50 yr and missing data on occupation, industry or SES	Industry/occupation code based on national census; assignments by industrial hygienist (IH) using database on chemical exposures and concentrations; potential misclassification into low and mod/hi, and exposure window during spermatogenesis for paternal exposure	Spontaneous abortion identified in hospital discharge register that occurred during a 2-yr period close to census	Adjusted for age, SES, & maternal exposure	Linear logistic regression adjusted for age, SES, and maternal exposure to reproductive hazards; risk odds ratio comparing exposed to unexposed	7,772 unexposed SA, 820 potential low, 139 moderate/high	**Spontaneous abortion**  Industry/occupation coding has low specificity; potential exposure misclassification and imprecise assignment of exposure period to period of spermatogenesis relevant to identified pregnancy
Saurel-Cubizolles et al. (1993) (cohort, retrospective) operating room nurses	Recruited operating room nurses at 18 hospitals (exposed) and randomly from nurses in other departments from same hospital (unexposed); data collection in both groups	Self-reported exposure (Y/N) to anesthetics, formol, & ionizing radiation during 1st trimester. No information on intensity and frequency.	Ectopic pregnancy: self-report by interview. Interviewed 1987-1988	Exposed and referent matched for age, duration of service, sex, occupation, and hospital. Formol exposure associated with exposure to anesthetics. No info on pelvic inflammatory disease but association with formaldehyde not likely.	Chi-square analysis for formol; no multivariate analyses	15 ectopic pregnancies of 734 pregnancies; 1 exposed case	**Ectopic pregnancy**  Small sample size and unadjusted analyses. No information on intensity and frequency of formaldehyde exposure (exposure misclassification—decreased sensitivity)

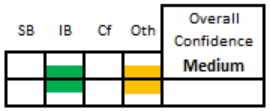
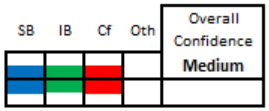
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	conducted the same						
Saurel-Cubizolles et al. (1994) (cohort, retrospective) operating room nurses	Recruited operating room nurses at 18 hospitals (exposed) and randomly from nurses in other departments from same hospital (unexposed); data collection in both groups conducted the same	Self-reported exposure (Y/N) to anesthetics, formol, & ionizing radiation during 1st trimester. No information on intensity and frequency.	Spontaneous abortion and birth defects (malformations ICD-9): self-report by questionnaire. First pregnancy in or after 1970; interviewed 1987–1988	Exposed and referent matched for age, duration of service, sex, occupation, and hospital. Formol exposure associated with exposure to anesthetics	Chi-square analysis for formol; no multivariate analyses	72 spontaneous abortions (9.4%); 22 pregnancies with birth defects (3.4%); 14 major malformations (2.2%)	**Spontaneous abortion and birth defects**  No information on intensity and frequency of formaldehyde exposure (exposure misclassification—decreased sensitivity). Possible confounding by other exposures and no adjustment (stronger associations observed for spontaneous abortion and anesthetics and ionizing radiation, but not all birth defects); no consideration of impact of gravidity on risk
Shumilina (1975) (cross sectional) cotton textile workers	Unable to assess; selection & response rate not reported	Range reported; sampling protocol not described; analyzed categories of textile finishers and sorted compared to saleswomen	Reproductive & pregnancy history including LBW. Gynecological exam and self-report; methods NR	Job demands among textile workers and referent (sales women) were different; shift work with standing and elevated ambient	Prevalence & SD; incomplete		**Reproductive disorders, and complications of pregnancy, low birth weight** 

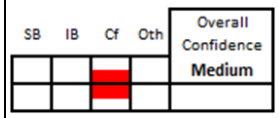
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
				temperature for exposed			**Not informative**; reporting deficiencies; potential confounding by conditions in the workplace
Steele and Wilkins (1996) (cohort, retrospective) veterinarians	Recruited from graduation rolls; 85% of eligible graduates. Graduated 1970–1980; survey 1987	Self-reported exposure (Y/N) to specific agents for specific jobs, defined exposed pregnancy if estimated time of conception occurred during years of job where exposure also was reported. 81% reported exposure to formaldehyde; no information on intensity or frequency of exposure.	Spontaneous abortion occurring for pregnancy started after graduation from veterinary college, < 20-wk gestation, self-reported	Compared exposed pregnancies to employed women who reported no exposure to formaldehyde or not employed during pregnancy. Adjusted for other risk factors, but not other workplace exposures	Unconditional logistic regression adjusting for maternal age, gravidity, previous SA, alcohol, and smoking. Also evaluated height, previous stillbirth, and previous induced abortions.	1,757 exposed pregnancies, 482 not exposed	**Spontaneous abortion**  No information on intensity and frequency of formaldehyde exposure which would likely be variable among veterinarians (exposure misclassification—decreased sensitivity). Adjustment for gravidity and previous spontaneous abortion may introduce bias.
Seitz and Baron (1990a) NIOSH Health Hazard Investigation (retrospective cohort) clothing manufacturer	Response: 98% of current employees, 18% of former employees employed 1984 or after. Possible survivor bias. Potential for	Air sampling 1987, full shift personal breathing zone for 5 task areas, 14 area samples full shift in several locations; perhaps not representative of earlier years;	Self-report, questionnaire, pregnancy while working at plant compared to employment at other locations or at home; miscarriage (not	Authors stated no differences among groups for other risk factors including smoking, alcohol, use of medications, and presence of diseases (diabetes)	Compared miscarriage and pregnancy outcomes by employment status when pregnancy occurred (employed at	Pregnancies among current: 19 at Rockcastle, 71 other,	**Miscarriage**  No comparison group (compared pregnancy

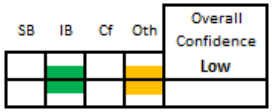
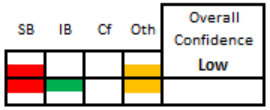
Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	selection bias for comparisons with pregnancy outcomes while at home (away from null); not a concern for comparisons with employment at other locations during pregnancy.	exposure range: TWA 0.17-0.57 mg/m^3; job status when pregnancy occurred.	defined), birth outcomes, self-report (questionnaire). Former workers sent questionnaire in 1984.		Rockcastle or other) or at home. RR (95% CI), Fisher's exact test	206 home	history during and not during job but could not account for gravidity in that kind of analysis). Limited exposure assessment for earlier years.
Stücker et al. (1993) (birth weight) (Stücker et al., 1990) (spontaneous abortion) (cohort, retrospective) nursing staff	Recruited all female daytime nursing staff, ≤ 45 yr old and currently working in selected units. 87% response among all daytime nursing staff	Current and previous jobs; self-report by interview; dates of each prior pregnancy and dates of occupational exposure to cytostatic drugs, anesthetic agents, and formaldehyde. Exposure based on exposure during or before the pregnancy. No information on intensity or frequency of exposure.	Self-report by interview (spontaneous abortion, birth weight, small for gestation age). Interview 1985–1986. Mean time since exposed and referent pregnancies, respectively, was 5 and 10 yrs (potential for differential recall and misclassification?)	Exposed and referent were all female day time nursing staff	No analyses were presented for spontaneous abortion. Linear regression for birth weight & formaldehyde association, adjusted for gestational age; not adjusted for other work exposures; other work exposures (quantitative results not reported, just reported as	348 births among formaldehyde exposed pregnancies; # of spontaneous abortions not reported	**Birth weight spontaneous abortion**  Inclusion of exposure before pregnancy of uncertain relevance for birth weight. No information on intensity and frequency of formaldehyde exposure (exposure misclassification—decreased sensitivity). Quantitative results not presented for formaldehyde for birth weight analysis; no

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
					"not significant")		results presented for spontaneous abortion analysis
Taskinen et al. (1994) (case-control) laboratory workers	Recruited from payrolls & union rolls, 82.4% response, reduced likelihood of selection bias; 2 referents per case with a live birth and no registered SA, 4 referents per congenital malformation case, study population restricted to age 20–34 yr, referents matched to case for age (24 mo) at conception and year at end of pregnancy	Self-report, focus on 1st trimester; exposed & frequency, reviewed by industrial hygienist; calculated exposure index based on reported quantity used, frequency (# hrs/d and # d/wk), and use of fume hood	Spontaneous abortion: hospital discharge register, 1973-1986	Smoking, alcohol and employment status considered a priori, plus other factors (parity, previous miscarriages, febrile diseases during pregnancy and used contraception) with OR > 1.5 or *p* value < 0.05; no other work exposures; possible confounding by xylene exposure, majority of formalin exposed also exposed to xylene (OR 3.1)	Conditional logistic regression adjusted for factors listed in confounding column	206 SA cases, 329 referents; 36 malformation cases, 105 referents	**Spontaneous abortion**  Adjustment for parity and previous miscarriage may introduce bias; lack of adjustment for xylene, an exposure associated with the spontaneous abortion and formalin exposure; evaluation of increasing frequency of use a strength.
Taskinen et al. (1999) (cohort, retrospective) woodworkers	Recruited from woodworker's union (not only current workers) reducing	TWA assigned using measurements and reported time at task, sampling protocol not	Pregnancies identified from national birth register 1985–1996; live birth.	FDR: Regression adjusting for several risk factors plus phenols, FDR for dusts & wood dusts	TTP: Discrete proportional hazards regression and likelihood ratio	Not exposed *N*=367 Low *N*=119	**Time-to-pregnancy** 

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	likelihood of survivor bias, 64% returned questionnaire; evaluated exposure response trend; period of recall 1–11 years. Not an optimal design for spontaneous abortion: women with no live births but at risk for spontaneous abortion were not included.	reported, JEM is a more robust exposure assessment; focused on 6 mos prior to pregnancy for TTP relevant exposure window; evaluated risk by glove use in high exposure group; Exposure range: 0.01–1.23 mg/m^3. Applied formaldehyde concentrations from a comparable workplace when data was missing (missing data was differential by exposure level; high 31%, moderate 61%, and low 46%)	Analysis limited to first pregnancy filling criteria; TTP (FDR): self-report (question: did woman get pregnant during first menstrual cycle when not using contraception? Second? Or how many mos/yrs?) Left censoring: excluded 38 pregnancies as a result of contraception failure & 28 whose TTP started before the first job in the branch. any previous SA: self-report	were > 1 in low exposure category & equal to 1 (1.02 & 0.93) in middle & high categories; SA: reported that other exposures were not associated	test, FDR (95% CI), adjusted for employment, smoking and alcohol consumption, irregular menstrual cycles, and # of children. Spontaneous abortion: Unconditional logistic regression, odds ratios, adjusted for age, employment, smoking and alcohol, # exposed cases not reported	Medium *N*=77 High *N*=39 52 spontaneous abortion cases (in women with same workplace as time-to-pregnancy analysis)	Expect some error in individual exposure assignments **Spontaneous abortion**  Exposures during critical exposure period(s) for spontaneous abortion were not estimated.; excluded women with no live birth (missing spontaneous abortions to women with no live births)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Wang et al. (2012) (cohort, retrospective) wood processing	100% of eligible recruited couples participated; did not describe recruitment or sampling frame; included if married males, Chinese Han ethnicity, had formaldehyde exposure for at least 24 mos; excluded couples with possible nonwork exposure to formaldehyde (i.e., newly remodeled homes), or wives with other exposures to reproductive toxicants & pregnancies prior to formaldehyde exposure	Measurements in factories, monitoring on 3 occasions during different periods; self-report of workplace, work tasks & hours/day exposed to formaldehyde; daily mean exposure = mean concentration multiplied by % of time exposed to formaldehyde (referenced (Taskinen et al., 1999). JEM is a more robust exposure assessment. Did not report formaldehyde estimates; relevant exposure period: gametogenesis	Prolonged time-to-pregnancy (> 12 mos), spontaneous abortion, birth outcomes (preterm birth, LBW, sex ratio, birth defects); semi-structured interview using questionnaire; data analysis for most recent pregnancy; potential under-ascertainment because interviewed male partners. Left censoring: 106 excluded because wife's pregnancy began before exposed employment	Exposed and referent matched on age, married men & from same area (salesmen and clerks); exposed and referent were of similar age, BMI, educational level, income, smoking, alcohol, frequency of intercourse. Confounding considered: age, BMI, education, income, smoking, alcohol, and frequency of intercourse. Adjusted for other risk factors but not for other work exposures (e.g., dust, phenols)	Logistic regression, paternal exposure risk; adjusted OR, 95% CI; compared low versus high formaldehyde exposed. Comparison of means (referent, low, and high) exposure, ANOVA; crude and adjusted regression coefficients and 95% CI; OR and 95% CI for abnormal sperm parameters; reported results of all analyses	Did not report # exposed and referent cases	**Time-to-pregnancy**  Exposure levels not reported (but robust assessment method). Dichotomized time-to-pregnancy in analysis (low sensitivity). **Spontaneous abortion birth defects**  Exposure levels not reported (but robust assessment method). Other workplace exposures in woodworking industry (solvents) have been associated with the spontaneous abortion but not accounted for; analysis of most recent pregnancy: possible selection for live births (time-lapse bias) and possible impact of gravidity

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
							on spontaneous abortion risk
Wang et al. (2015) (cohort, retrospective) wood processing, 7 industrial sites	Recruited men aged 23–40 yrs of age, Chinese Han ethnicity, and formaldehyde exposed at least 24 mos. Excluded men who lived in newly built or recently decorated house, men with genital malformations or other chronic diseases. Comparison: age-matched male Han population volunteers living in same area (salesmen and clerks) not	Referenced Wang et al. (2012); sampling: 25-min samples at 3 times on one workday, same day as investigation . Exposure information based on workplace, work tasks, work duration and time. Exposure index based on formaldehyde concentration (mean of 3 samples) times exposed work time during work day times exposure duration (years). Two categories with cutpoint at median.	Semi-structured interview using questionnaire; no change in lifestyle or environments 6 mo prior to semen collection; genital examination. Semen sample within 2 wks of exposure sampling, after a 2–7 d abstinence. Semen analysis within 60 min by two technicians using same apparatus (computer assisted semen analysis), blinded. Parameters: semen volume,	Addressed via design, sex, SES, education, age. Variables included in models: age, body mass index, education, income, smoking, drinking, and abstinence duration. No evaluation of other organic solvents such as phenol or wood preservatives.	Multiple linear regression of ln-transformed semen parameters and logistic regression of abnormal semen parameters; reported results for all parameters analyzed	124 (62.3%) recruited, eligible and agreed to participate. 75 of 199 eligible refused to provide sample. No data for 10, *N*=114 *N*=81 referents (40.5% of eligible), no	 Other workplace exposures in woodworking industry (solvents) have been associated with sperm motility but not accounted for; however otherwise strong design and analysis, including evaluation of increasing exposure-response relationship.

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	exposed to formaldehyde or other reproductive toxicants.	Concentrations: Exposed 0.22–2.91 mg/m^3, exposure index 4.54–195.08, median 56.55; referent 0–0.02 mg/m^3.	sperm concentration, total sperm count, sperm progressive motility and total sperm motility; kinematic parameters (WHO, 2010), velocity, linearity, displacement measures.			semen data for 5, *N*=76	
Ward et al. (1984) (cross-sectional) autopsy service	Groups similar: exposed and referent all from university (exposed = autopsy service; referent = other medical branches)	Reported ranges for TWA and concentration; area and personal breathing zone. Exposure range: TWA 0.75–1.62 mg/m^3	Sperm abnormalities assessed every 2–3 months (3 samples collected for standard sperm parameters); hand scoring of morphology (no QC data)	Matched on sex, age, tobacco, alcohol, and recreational drug use	No statistical analyses; EPA could compare prevalence	11 men per exposure group	**Sperm parameters**  Small sample size; uncertainty regarding reliability of morphology scoring
Zhu et al. (2005) (pregnancy cohort) laboratory work	Danish National Birth Cohort, 30-40% of all pregnancies, first pregnancy and laboratory technician (hospital, university, medical industry,	Self-report at gestational weeks 12–25 (median 17 wks), laboratory work processes during pregnancy and 3 mos before conception; JEM exposure index: exposure level (low	Self-report of TTP (0–2 months, 3–5 months, 6–12 months, >12 months); fecundability ratio	Demographic characteristics of laboratory technicians and teachers comparable (maternal age, gravidity, history of spontaneous abortion, smoking, alcohol, BMI, paternal	Fecundability ratios analyzed within the exposed group (exposure index 1–5 vs >=6) using discrete-time survival analysis; adjusted for	Exposed *N*=829, referent *N*=6,250	**Time-to-pregnancy**  Categorized time-to-pregnancy (decreased precision), missed pregnancies that ended before 1st interview.

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	food industry or public services), 77.5% initial cohort; referent teachers, 73.9% initial cohort; entered cohort at weeks 12–25 (median 17)	or medium assigned to work process by study investigators) times frequency of contact. Formaldehyde: Low: processed human blood or tissues, worked with experimental animals or microorganisms; Medium: prepared slides for microscopy. Exposure index did not include use of protective measures (40–64% used exhaust/flow bench). Exposure tool was not validated for formaldehyde		job). Possible confounding by other exposures in lab	covariates listed in confounding column		Variation in probability or intensity of formaldehyde exposure possible for work processes across different types of labs, did not account for large proportion of participants who used protective measures to prevent inhalation exposure. JEM was not validated for formaldehyde.
Zhu et al. (2006) (cohort study) laboratory work	Members of the Danish National Birth Cohort, 30–40% of all pregnancies, first pregnancy and laboratory technician (hospital,	Self-report at gestational weeks 12–25 (median 17 wks), laboratory work processes during pregnancy and 3 mos before conception; JEM exposure index: see	Birth outcomes: preterm birth, small for gestational age, major malformations	Demographic characteristics of laboratory technicians and teachers comparable (maternal age, gravidity, history SA, smoking, alcohol, BMI, paternal job).	Cox regression within the exposed group (exposure index 1–5 vs ≥6), hazard ratios for fetal loss and malformations;	Late fetal loss: exposed 9, unexposed 106; preterm birth: exposed	**Preterm birth** **small for gestational age** **major malformations** SB IB Cf Oth Overall Confidence Low ↓ Variation in probability or intensity of formaldehyde

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	university, medical industry, food industry or public services), 95% of eligible; referent teachers, 95% of eligible	Zhu et al. (2005) above		Possible confounding by other exposures in lab	logistic regression, odds ratios for other outcomes; adjusted for covariates listed in confounding column	41, unexposed 317; SGA: exposed 80, unexposed 700; major malformations: exposed 56, unexposed 379	exposure possible for work processes across different types of labs, did not account for large proportion of participants who used protective measures to prevent inhalation exposure. JEM was not validated for formaldehyde.

Animal Studies

Only in vivo inhalation exposure studies are used for hazard identification and dose-response assessment. These studies were conducted in inhalation chambers under controlled experimental conditions. Studies that exposed animals to formaldehyde via other routes were not included because they are expected to result in significant distribution of formaldehyde past the portal of entry, which does not occur to a great extent with inhalation exposures.

Evaluation of experimental studies

The experimental animal studies were each assigned confidence ratings of: High, Medium, or Low Confidence, and "Not Informative" based on an evaluation of the experimental details for each study and an expert judgement related to predefined criteria for (1) exposure quality, (2) test animals, (3) study dosing, (4) endpoint evaluation, and (5) data considerations and statistical analysis (described in Appendix A.1.1.). The studies designated as "Not informative" included those with documented chemical co-exposure (in addition to inhaled formaldehyde) that might have compromised the developmental or reproductive outcomes evaluated, or those that did not present sufficient information to fully assess the study methods or test results for assessments critical to study interpretation. The studies judged to be "Not informative" are not discussed in the Toxicological Review.

Due to the known developmental hazard of methanol, studies failing to use an appropriate test article (see Appendix A.1.2) or that did not provide a full characterization of the test substance were automatically assigned a rating of "Low Confidence", and may be deemed "Not Informative" if additional study limitations are identified.

In addition to the general criteria discussed in Appendix A.1.1., considerations specific to the evaluation of potential developmental or reproductive system effects were also evaluated:

- The potential contribution of species and strain-related differences in reproductive schedules and outcome sensitivity were considered. The age of the animals, life stage, and critical windows of exposure and assessment were evaluated for potential influence on study results.

- The power of the study (group size, and sample size for specific endpoints) was considered. Typical standards for guideline developmental and reproductive toxicity studies (i.e., preferably at least 20 dams/group) may not always be relevant to the endpoint-targeted studies published in the literature. Negative studies with less than 10 test subjects per group were considered to be "Low confidence."

- Random assignment of animals to exposure groups or to a specific assessment subgroup, "blinding" to study group, or other procedures that were applied with the intent of mitigating potential bias was preferred.

- Studies were examined for evidence of severe overt toxicity in parental animals or offspring, and the potential influence of maternal toxicity on fetal or postnatal offspring outcomes was considered.

- In general principle, methodologies used to assess specific endpoints were evaluated in comparison to published standards, guidance, and/or guidelines, although developmental and reproductive toxicity database contained no guideline studies conducted under strict Good Laboratory Practice regulations.

- The intent and focus of the study was considered when evaluating limitations in study design because it is recognized that not all available studies are designed to screen for a wide array of developmental or reproductive outcomes. Sometimes only part of the data from a study might be deemed adequate.

- Presentation of detailed methodological information was necessary, given the complexity of studies that assess developmental and reproductive outcomes, and the potential for small variation in study design to have an impact on study outcome.

- Inclusion of adequately characterized quantitative and/or qualitative data to support study conclusions was considered critical to the evaluation of study quality. The report was examined to determine if the litter was considered the primary unit of analysis for offspring data.

Additional considerations that might influence the interpretation of the usefulness of the studies during the hazard synthesis are noted, including limitations such as a short exposure duration or the use of only one test concentration or concentrations that are all too high or too low to provide a spectrum of the possible effects, as well as study strengths such as very large sample sizes or particularly robust endpoint protocols; however, this information typically did not affect the study evaluation decisions.

If the conduct of the experimental feature was considered to pose a substantial limitation that is likely to influence the study results, the cell is shaded gray; a "+" is used if potential issues were identified, but these are not expected to have a substantial influence on the interpretation of the experimental results; and a "++" denotes experimental features without limitations that are expected to influence the study results. Specific study details (or lack thereof) which highlight a limitation or uncertainty in answering each of the experimental feature criteria are noted in the cells. For those experimental features identified as having a substantial limitation likely to influence the study results, the relevant study details leading to this decision are bolded. Studies are organized according to the general outcomes evaluated (i.e., gestation exposures and developmental outcomes and reproductive outcomes) and then listed alphabetically.

Table A-93. Study quality evaluation of developmental and reproductive toxicity animal studies

	Experimental Feature Categories *The study detail(s) leading to identification of a major (bolded) or minor (italicized) experimental feature limitation is indicated*					
	Exposure Quality	**Test Subjects**	**Study Design**	**Endpoint Evaluation**	**Data Considerations & Statistical Analyses**	**Overall Confidence Rating Regarding the Use for MOA** (Main limitations**)** Expert judgement based on conclusions from evaluation of the 5 experimental feature categories
Criteria relevant to evaluating the experimental details within each experimental feature category	Exposure quality evaluations (see A.5.1) are summarized below; "++": robust; "+": adequate; and shaded box: poor; relevance of the tested exposure levels is discussed in the hazard synthesis	The species, sex, strain, and age are appropriate for the endpoint(s); sample size provides reasonable power to assess the endpoint(s); overt systemic toxicity is absent or not expected, or it is accounted for; group allocations can be inferred as appropriate	A study focus was developmental or reproductive system effects; the exposure regimen is informative for the tested endpoint(s); manipulations other than formaldehyde exposure are adequately controlled[i]	Endpoint evaluates a mechanism relevant to humans[ii]; protocols are complete, sensitive, discriminating, and biologically sound; experimenter bias minimized	Statistical methods, group comparisons, and data presentation (including variability) are complete, appropriate, and discerning; selective reporting bias avoided	
Gestation Exposures and Developmental Outcomes[iii]						
Al-Saraj (2009)	Test article = formalin; **co-exposure with ivermectin** (anhelmintic)	+ 7 control does and 26 FA-exposed does; *strain NR*	**Gestation day not standardized via cesarean section; detailed offspring evaluation methods not provided**	**Only external examination; no visceral or skeletal evaluation of newborn kits**	**Exposure during gestation not well-characterized; dose-dependent data in dams and offspring not shown. Litter incidences of external findings not provided; major confounding factor: co-exposure with ivermectin, a known**	Not informative (Co-exposure to ivermectin)

					developmental toxicant in rabbits	
Gofmekler (1968)	**Test article NC**; *generation method,* analytical method and concentrations, chamber type NR; *exposure regimen poorly characterized*	+ *N* = 3 males and 12 females/group; *source and strain NR*	+ Limited study design focused on offspring growth (body weight and organ weight)	+ *Methods were poorly described* but appeared appropriate for the evaluation of offspring growth	**Mean body and organ weight data reported, but no variance provided; statistical methods not described although statistical analysis was conducted. Age at assessment of offspring NR; reproductive (maternal and litter) data not provided; overall limited data reporting.**	**Low** (Test article NC, exposure generation, animal strain/source NR; limited description of methods; limited reporting)
Gofmekler and Bonashevskaya (1969)	**Test article NC**; generation method, analytical method and concentrations, chamber type, exposure regimen NR	+ *N* = 12/group; *source and strain NR*	+ Limited study design focused on developmental anomalies, offspring reproductive organ weights, and histopathology	+ Methods were poorly described but appeared appropriate for the evaluation of.	**Report contained only verbal summary of findings. No quantitative data were included in the paper**	Not informative (Test article NC, exposure generation, animal strain/source NR; limited description of methods; limited reporting)
Guseva (1973a)	**Test article NC**; *generation method,* analytical concentrations *NR; chamber type NC*; **co-exposure with formalin in drinking water**	***N* = 4/group**; *source and strain NR*	+ Limited study design focused on reproductive function, developmental anomalies and postnatal maturation; gonadotropic response to pituitary emulsions, and testicular nucleic acids	+ *Methods were poorly described* but some appeared appropriate for the evaluation of reproductive function, developmental anomalies and postnatal maturation; gonadotropic response assay was not a standard testing paradigm	**Only nucleic acid quantitative data (mean and variance) were reported; all other results were described verbally; statistical methods not described although statistical analysis was conducted**	Not informative (Test article NC; oral co-exposure with formalin; low *N*; some experimental methods and data NR)

Kitaev et al. (1984)[d]	**Test article NC**; generation method, analytical concentrations NR; chamber type NC	+ *N* = 5–9/group; *source NR*	+ Limited study design focused on early embryonic development, organ weights, and hormone measures; time of day the hormone measures were taken NR	+ *Methods were poorly described* but appeared appropriate for the evaluation of early embryonic development, organ weights, and hormone measures	+ Group mean data and variance presented for embryos/rats; variance shown in graphics for organ weights and hormone measures; statistical methods not described although statistical analytical results were described in text. Statistical significance NR for some embryonic outcomes; relative organ weight and hormone measure graphs appeared to be hand-drawn	**Low** (Test article NC; limited description of methods)
Kum et al. (2007a)	**Test article = formalin**; generation method, analytical concentrations NR	+ *N* = 6/group; *source NR*	+ Limited study design focused on embryonic and early postnatal body and liver weights and MOA (redox enzymes)	+ *Methods were poorly described* but appeared appropriate for the evaluation of embryonic and early postnatal body and liver weights	+ Group mean data and variance presented; *maternal toxicity not reported*	Low (Formalin; limited description of methods; maternal tox NR)
Martin (1990)	++ Test article = paraformaldehyde; well characterized exposure methods	+ *N* = 25 dams/group; *source NR*	+ Study design described as a "teratology study" although few details were provided	**Methods were not described;** endpoints listed in the statistical methods section appeared appropriate for a screening level evaluation of developmental toxicity	+ *Inadequate reporting of methods and quantitative results. No group mean data were presented*	**Low** (Inadequate reporting of methods and quantitative results)
Monfared (2012)	**Test article NC**; generation method, analytical methods and concentrations NR	++ *N* = 10 dams/group; strain and source were reported	+ Limited study design focused on placental weight, histopathology,	++ Methods were appropriate for the evaluation of placental weight,	+ Group mean placental weight data and variance presented; photomicrographs	**Low** (Test article NC; maternal tox: NR)

			and ultrastructural pathology	histopathology, and ultrastructural pathology	provided; *maternal toxicity not reported*	
Pushkina et al. (1968)	**Test article NC**; generation method, analytical method and concentrations, chamber type, exposure regimen NR	+ *N* = 10 females/group; *strain NR*	+ Limited study design focused on ascorbic acid levels in dams, fetuses, and placentas	**Limited methodological information provided**	+ Group mean ascorbic acid levels and variance presented; *statistical methods not described* although statistical analytical results were noted in table	Not informative (Experimental methods NR)
Saillenfait et al. (1989)	**Test article = formalin** with 10% methanol; well-characterized exposure methods	++ *N* = 25 dams/group; strain and source provided	++ Study design was equivalent to a guideline prenatal developmental toxicity study	++ Methods well described and appropriate for a screening level evaluation of developmental toxicity.	++ Group incidence and mean/variance data presented	**Low** (Formalin)
(Sanotskii et al., 1976)	**Test article NC**; generation method, analytical method and concentrations NR; chamber type NC	*N* = 334 total females (**females/group NR**); strain and source NR	**Limited study design only evaluated pregnant vs. nonpregnant dams (did not evaluate reproductive or fetal parameters)**	**Limited methodological information provided**	**Inadequate reporting of methods and results (no primary or mean data presented); statistical methods not described although statistical analytical results were noted in text**	Not informative (Experimental methods and data NR)
Senichenkova (1991a)	**Test article NC**; generation method, analytical method and concentrations NR; chamber type NC	*N* = 137 total dams (**dams/group NR**); *strain and source NR*	+ Study design focused on in utero developmental outcomes (mortality, growth, visceral, skeletal outcomes), select open field neurotoxicity measurements in juveniles, and blood acid-base status	+ *Limited methodological information* provided for tests conducted; apparent methods appropriate for the evaluation of in utero developmental outcomes.	+ Group mean and variance data presented; *maternal toxicity not reported*; statistical methods not described although statistical analytical results were noted in tables	**Low** (Test article NC; exposure generation, animal strain/source, # dams/group, maternal tox NR; limited description of methods)

Senichenkova, 1996, 667201@@author-year}	**Test article NC**; generation method, analytical method and concentrations NR; chamber type NC	*N* = 254 total dams (**dams/group NR**); *strain and source NR*	+ Control group co-exposure to ethanol; limited study design focused on in utero developmental outcomes (external anomalies and skeletal delays) and blood acid-base status	+ Limited methodological information provided for tests conducted; apparent methods appropriate for the evaluation of in utero developmental outcomes.	+ Group mean and variance data presented; statistical methods not described although statistical analytical results were noted in tables; *maternal toxicity not reported*	Low (Test article NC; exposure generation, animal strain/source, # dams/group, maternal tox NR; limited description of methods)
Sheveleva (1971)	**Test article NC**; generation method, analytical method NR	+ *N* = 15 dams/group for C-section, 6 dams/group for delivery; *strain and source NR*	+ Limited study design focused on developmental parameters, body weight spontaneous mobility, temperature, and hematology parameters	+ Limited methodological information provided for tests conducted; apparent methods appropriate for the evaluation of developmental parameters.	+ Group mean and variance data presented; statistical methods not described	**Low** (Test article NC; exposure generation, animal strain/source NR; limited description of methods)
Reproductive Outcomes						
(Appelman et al., 1988**)**	**++** Test article = paraformaldehyde; well characterized exposure methods	++ *N* = 40 males/group; test animals adequately characterized	++ Study design focused on comparison of subchronic or chronic exposures to rats with undamaged or clinically damaged nasal mucosa; extensive tissue evaluation	**No indication if histopathology was performed on male reproductive organs**	**Quantitative testes weight data were not presented in the study results. No histopathology findings for male reproductive organs were reported**	**Low** (No indication if histopathology performed on male repro organs; quantitative testes weights not presented)
Golalipour et al. (2007)	**Test article NC**; generation method NR; **open air exposures** (i.e., not a controlled chamber study)	***N* = 4 males/group**; test animals adequately characterized	+ Limited study design focused on testis toxicity	++ Methods were appropriate for the evaluation of testis toxicity.	++ Group mean data and variance presented	**Low** (Test article NC; open air exposures; N = 4/group)

Han et al. (2015)	**Test article NC**; *generation method,* analytical method and concentrations *NR, static chamber type*	++ *N* = 10 males/group; test animals adequately characterized	+ Limited study design focused on testis toxicity and MOA	+ Methods were appropriate for the evaluation of testis toxicity.	+ Group mean testis weight and seminiferous tubule diameter data reported but *variance not presented; quantitative microscopy findings not presented*	**Low** (Test article NC; exposure generation NR; static chamber used; limited reporting of study results and group data)
Maronpot et al. (1986)	**Test article = formalin**; well-characterized exposure methods	++ *N* = 10/sex/group; test animals adequately characterized	++ Subchronic study with limited in-life observations and extensive postmortem evaluation	++ Methods were appropriate for a screening level evaluation of general toxicity following subchronic exposure; no special emphasis on reproductive organs	+ *Selected incidence data presented (survival, histopathology); mean body weight data did not include variance; no indication of statistical data analysis*	**Low** (Formalin; limited reporting of methods and results)
Ozen et al. (2002)	**++** Test article = paraformaldehyde; well characterized exposure methods	++ *N* = 7 males/group; test animals adequately characterized	+ Limited study design focused on testis toxicity and MOA	++ Methods were appropriate for the evaluation of testis toxicity	++ Group mean data and variance presented	**High** (None)
Ozen et al. (2005)	+ Test article = paraformaldehyde; *analytical concentrations NR*	++ *N* = 6 males/group; test animals adequately characterized	+ Limited study design focused on testis toxicity (includes Bouins fixation of testes)	++ Methods were appropriate for the evaluation of testis toxicity	++ Group mean data and variance presented	**High** (None)
Sapmaz et al. (2018)	**++** Test article = paraformaldehyde; well characterized exposure methods	+ *N =7 adult males; strain provided; source not identified*	+ Limited study design focused on testis toxicity and biomarkers of oxidative stress; only one paraformldehyde test group	++ Methods were appropriate for the evaluation of testis toxicity	++ Group mean data and variance presented	**Medium** (Inadequate information for quantitative analysis of histopathology data)

Sarsilmaz et al. (1999)	+ Test article = paraformaldehyde; analytical concentrations NR	++ *N* = 10 males/group; test animals adequately characterized	+ Limited study design focused on testis toxicity	++ Methods were appropriate for the evaluation of testis toxicity	+ Group mean data and variance presented; *unable to determine what the reported SD represents for Leydig cell numbers*	**Medium** (Inadequate information for quantitative analysis of histopathology data)
Vosoughi et al. (2013); Vosoughi et al. (2012)	**++** Test article = paraformaldehyde; well characterized exposure methods; analytical concentrations reported	++ *N* = 12 males/group; test animals adequately characterized	+ Limited study design focused on testis toxicity, sperm measures, and hormone (LH, FSH, T) levels	++ Methods were appropriate for the evaluation of testis toxicity, sperm measures, and hormone levels (LH, FSH, T)	++ Group mean data and variance presented	**High** (None)
Wang et al. (2013)	**Test article NC**; generation method, analytical method and concentrations NR, static chamber type	++ *N* = 10 females/group; test animals adequately characterized	+ Limited study design focused on ovarian toxicity, estradiol (E2) levels, and MOA	++ Methods were appropriate for the evaluation of ovarian toxicity and E levels	++ Group mean data and variance presented (graphically) for E2 levels and ovarian weights	**Low** (Test article NC)
(Woutersen et al., 1987)	++ Test article = paraformaldehyde, generation method, analytical methods and concentrations reported, dynamic whole-body chamber	**++** *N* = 40/sex/group; test animals adequately characterized	++ 13-week subchronic study	Report indicates that testes and ovaries were weighed at necropsy; **no indication if histopathology was performed on male or female reproductive organs**	**Quantitative reproductive organ weight data were not presented in the study results. No histopathology findings for reproductive organs were reported**	**Low** (Limited methods; no data presented)
Xing et al. (2007)	**Test article NC**; *generation method,* analytical	++ *N* = 12 males and 24 females/group; test	+ Limited study design focused on sperm	++ Methods were appropriate for the evaluation of sperm	++ Adequate reporting of reproductive outcome	**Low** (Test article NC; exposure

	method and concentrations, *chamber type NR*	animals adequately characterized	morphology, reproductive success, and micronucleus assay	morphology and reproductive outcome.	results (group incidence and mean data with variance). Micronucleus data not presented.	generation, strain NR; high exposure levels)
Zhou et al. (2006)	**Test article NC**; *generation method* and analytical concentrations *NR, static chamber type*	++ *N* = 10 males/group; test animals adequately characterized	+ Limited study design focused on testes weight and histopathology, sperm measures, and MOA; co-exposure of one FA-treated group with vitamin E to assess mediation effects	++ Methods were appropriate for the evaluation of testes weight and histopathology, and sperm measures	++ Group mean data and variance presented (graphically for testes weights); appropriate statistical analysis of data. Vitamin E co-exposure group not included in dose-response assessment for FA outcomes	**Low** (Test article NC, exposure generation NR; static chamber used)
Zhou et al. (2011a)	**Test article NC**; generation method, analytical method and concentrations NR; *static chamber type, exposure regimen poorly described*	++ *N* = 10 males/group; test animals adequately characterized	+ Limited study design focused on testes and epididymal weight and histopathology, sperm measures, testosterone (T) levels, and MOA	++ Methods were appropriate for the evaluation of testes and epididymal weight and histopathology, sperm measures, and T levels	++ Group mean data and variance presented (graphically for T levels)	**Low** (Test article NC; exposure generation NR; static chamber used)
Zhou et al. (2011b)	**Test article NC**; generation method, analytical method and concentrations NR; *static chamber type, exposure regimen poorly described*	++ *N* = 12 males/group; test animals adequately characterized	+ Limited study design focused on epididymal weight and histopathology, sperm measures, and MOA	++ Methods were appropriate for the evaluation of epididymal weight, histopathology, and sperm measures	++ Group mean data and variance presented (graphically)	**Low** (Test article NC; exposure generation NR; static chamber used)

NR = Not Reported; NC = Not Characterized
Gradations of sufficiency based upon described criteria: ++ = meets sufficiency criteria; + = meets some sufficiency criteria

A.5.9. Carcinogenicity: Respiratory Tract, Lymphohematopoietic, or Other Cancers

Systematic identification and evaluation of the literature database on studies examining the potential for carcinogenicity following formaldehyde exposure was performed separately for the following: (1) human studies of respiratory tract, lymphohematopoietic, or other cancers; (2) experimental animal studies of respiratory tract (nasal) cancers; and (3) experimental animal studies of LHP cancers. This section is organized accordingly.

Literature Search

Studies in Humans

A systematic evaluation of the literature database on studies examining the potential for cancer in humans in relation to formaldehyde exposure was initially conducted in October 2012, with yearly updates to September 2016 (see A.5.1 for searches through 2016; see Appendix F for details on a separate Systematic Evidence Map that updates the literature from 2017–2021 using parallel approaches). The search strings used in specific databases are shown in Table A-94**.** Additional search strategies included:

- Review of reference lists in the articles identified through the full screening process.
- Review of reference lists in the 2010 draft Toxicological Review for Formaldehyde (U.S. EPA, 2010), the ATSDR toxicological profile of formaldehyde (ATSDR, 1999), and the NTP report on carcinogens background document for formaldehyde (NTP, 2010).
- Review of references in 11 review articles relating to formaldehyde and cancer, published in English, identified in the initial database search.

Relevant studies were separated into upper respiratory tract (URT) cancers, lymphohematopoietic (LHP) cancers, and other cancers (including brain, lung, pancreatic, etc.). Inclusion and exclusion criteria used in the screening step are described in Table A-95.

Multiple review articles and meta-analyses have examined the epidemiologic evidence informing potential associations between formaldehyde and cancer endpoints (e.g., e.g., Bachand et al., 2010; Zhang et al., 2009; Bosetti et al., 2008; Collins and Lineker, 2004; Collins et al., 2001; Ojajärvi et al., 2000; Collins et al., 1997; Blair et al., 1990). The vast majority of studies focused on cancers of the URT and LHP system. Other cancers endpoints reported in the literature include bladder, brain, colon, lung, pancreas, prostate, and skin. However, aside from cancer of the brain and lung, few studies showed any evidence of increased risks. Given the large number of studies available on URT and LHP cancers, the other endpoints were not included in the hazard evaluation. As numerous studies reported data on cancers of the brain or lung, a summary of the available studies for each of these endpoints is provided in Appendix A.5.9 for information; however, a cursory review of the available studies did not suggest any consistent association with formaldehyde exposure and, as such, these endpoints were also not formally reviewed.

For the hazard evaluation, the URT cancer endpoints were restricted to specific cancers (i.e., nasopharyngeal cancer, sinonasal cancer, cancers of the oro- and hypopharynx, and laryngeal cancer). The specific LHP cancers that were formally reviewed were Hodgkin lymphoma, multiple myeloma, myeloid leukemia, lymphatic leukemia. Non-Hodgkin lymphoma is a nonspecific grouping of dozens of different lymphomas and classification systems for specific subtypes have changed over time, complicating the synthesis of study results for this cancer type. If formaldehyde is associated with particular non-Hodgkin lymphoma subtypes, then these studies might be not sensitive enough to detect an association. As review articles and a cursory review of the available did not suggest an association between formaldehyde exposure and non-Hodgkin lymphoma and, as such, this endpoint was not formally reviewed.

After manual review and removal of duplication citations, the 624 articles identified from database searches were initially screened within an EndNote library for relevance; title was considered first, and then abstract in this process. Full text review was conducted on 271identified articles. The search and screening strategy, including exclusion categories applied and the number of articles excluded within each exclusion category, is summarized in Figure A-37.

Based on this process, **59 studies** were identified and evaluated for consideration in the Toxicological Review.

Table A-94. Summary of search terms for carcinogenicity in humans

Database, search date	Terms
PubMed No date restriction	"formaldehyde"[Majr] AND ("neoplasms"[All Fields] OR "cancer"[All Fields] OR "leukaemia"[All Fields] OR "leukemia"[All Fields] OR "multiple myeloma"[All Fields] OR ("multiple"[All Fields] AND "myeloma"[All Fields]) OR "multiple myeloma"[All Fields] OR "myeloma"[All Fields] OR "lymphoma"[All Fields] OR "nasopharyngeal neoplasms"[All Fields] OR ("nasopharyngeal"[All Fields] AND "neoplasms"[All Fields]) OR "nasopharyngeal neoplasms"[All Fields] OR ("nasopharyngeal"[All Fields] AND "cancer"[All Fields]) OR "nasopharyngeal cancer"[All Fields] OR ("sinonasal" [All Fields] AND "neoplasms"[All Fields]) OR "neoplasms"[All Fields] OR "cancer"[All Fields] OR "oropharyngeal neoplasms"[All Fields] OR ("oropharyngeal"[All Fields] AND "neoplasms"[All Fields]) OR "oropharyngeal neoplasms"[All Fields] OR ("oropharyngeal"[All Fields] AND "neoplasms"[All Fields]) OR "laryngeal neoplasms"[All Fields] OR ("laryngeal"[All Fields] AND "neoplasms"[All Fields]) OR "laryngeal neoplasms"[All Fields] OR ("laryngeal"[All Fields] AND "cancer"[All Fields]) OR "laryngeal cancer"[All Fields]) AND (Epidemiol*[All Fields] OR "Case-control studies"[All Fields] OR "Cohort studies"[All Fields] OR "Follow-up studies"[All Fields] OR "Risk factors"[All Fields])
Web of Science No date restriction Lemmatization "off"	TS=formaldehyde AND (TS=neoplasms OR TS=cancer OR TS=leukaemia OR TS=leukemia OR TS=multiple myeloma OR (TS=multiple AND TS=myeloma) OR TS=multiple myeloma OR TS=myeloma OR TS=lymphoma OR TS=nasopharyngeal neoplasms OR (TS=nasopharyngeal AND TS=neoplasms) OR TS=nasopharyngeal neoplasms OR (TS=nasopharyngeal AND TS=cancer) OR TS=nasopharyngeal cancer OR (TS=sinonasal AND TS=neoplasms) OR TS=oropharyngeal neoplasms OR (TS=oropharyngeal AND TS=neoplasms) OR TS=oropharyngeal neoplasms OR (TS=oropharyngeal AND TS=neoplasms) OR TS=laryngeal neoplasms OR (TS=laryngeal AND TS=neoplasms) OR TS=laryngeal neoplasms OR (TS=laryngeal

Database, search date	Terms
	AND TS=cancer) OR TS=laryngeal cancer) AND (TS=Epidemiol* OR TS=Case-control studies OR TS=Cohort studies OR TS=Follow-up studies OR TS=Risk factors)
ToxNet (Toxline and DART) No date restriction English, not including PubMed	Formaldehyde AND (neoplasms OR neoplasms OR cancer OR leukaemia OR leukemia OR "multiple myeloma" OR (multiple AND myeloma) OR myeloma OR lymphoma OR "nasopharyngeal neoplasms" OR (nasopharyngeal AND neoplasms) OR "nasopharyngeal neoplasms" OR (nasopharyngeal AND cancer) OR "nasopharyngeal cancer" OR (sinonasal AND neoplasms) OR "oropharyngeal neoplasms" OR (oropharyngeal AND neoplasms) OR "oropharyngeal neoplasms" OR (oropharyngeal AND neoplasms) OR "laryngeal neoplasms" OR (laryngeal AND neoplasms) OR "laryngeal neoplasms" OR (laryngeal AND cancer) OR "laryngeal cancer") AND (Epidemiol* OR "Case-control studies" OR "Cohort studies" OR "Follow-up studies" OR "Risk factors"))

Table A-95. Inclusion and exclusion criteria for evaluation of studies of cancer in humans

	Included	Excluded
Population	• Human	• Animals
Exposure	• Exposure assessment for formaldehyde • Industries or occupations known to involve exposure to formaldehyde	• Not formaldehyde • Outdoor formaldehyde exposure
Comparison		• Case reports
Outcome	• Nasopharyngeal cancer • Sinonasal cancer • Cancers of the oro- and hypopharynx • Laryngeal • Specific lymphohematopoietic cancers (i.e., Hodgkin lymphoma, multiple myeloma, myeloid leukemia, lymphatic leukemia	• Bladder, colon, pancreas, prostate, and skin • Brain and lung cancer studies were initially included but were subsequently excluded from the systematic review • Non-Hodgkin lymphoma
Other		• Reviews, reports, letters, commentaries, meeting abstracts, methodology papers

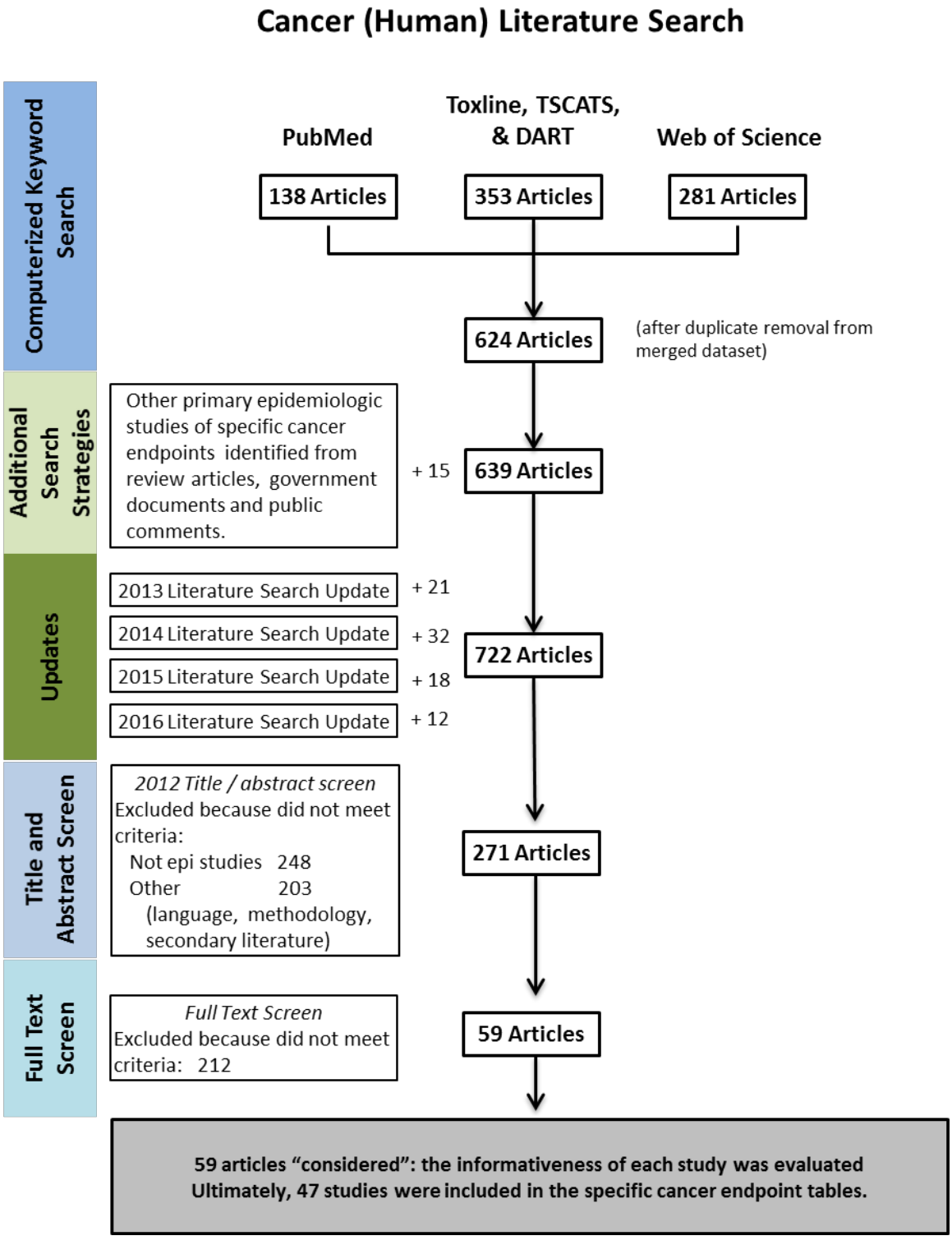


Figure A-37. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and upper respiratory or lymphohematopoietic cancers in humans through 2016 (see Appendix F for details on the systematic evidence map updating the literature through 2021).

Studies in Animals

Based on the available evidence, separate systematic literature evaluations were conducted as follows: (1) literature related to respiratory tract cancers and (2) literature related to LHP cancers. These searches were initially conducted in October 2012, with yearly updates (see Section A.1.1 for searches through 2016; see Appendix F for details on a separate Systematic Evidence Map that updates the literature from 2017–2021 using parallel approaches). Similar to the evidence in humans described above, the animal evidence for cancers other than those of the respiratory tract and the LHP system were not systematically identified and reviewed; rather, these observations (as identified through other, health effect-specific searches) were summarily described. For the respiratory tract, the strategies are summarized in figure format (see Figures A-38); the search strings used in specific databases are shown in table format (see Tables A-96), with additional details of the process described below. For LHP cancer searches, the strategies are summarized in figure format (see Figures A-39); the search strings used in specific databases are shown in table format (see Tables A-98), with additional details of the process described below.

Respiratory tract (i.e., nasal) cancers in animals

A systematic evaluation of the literature database on studies examining the potential for respiratory tract cancers following formaldehyde exposure was conducted through September 2016. This search strategy is summarized in Figure A-38; the search strings used in specific databases are shown in Table A-96 with additional details of the process described below, and the criteria used for inclusion and exclusion of studies during screening described in Table A-97.

Table A-96. Summary of search terms for respiratory tract cancers in animals

Database, search date	Terms
PubMed 04/15/2013 No date restriction	Formaldehyde [majr] AND (animal OR rodent OR rat OR mouse OR hamster) AND (nasal OR nose OR buccal OR larynx OR lung OR mouth OR pharynx OR sinus OR trachea) AND (cancer OR dysplasia OR neoplasia OR tumor OR carcinoma OR polyp OR cytotoxicity OR neoplastic OR promoter OR pathology OR toxicity) NOT (formalin test OR formaldehyde fixation OR formalin fixed OR formaldehyde fixed OR formalin-induced OR formaldehyde-induced)
Web of Science 03/08/2013 No date restriction Lemmatization "off"	Formaldehyde (title) AND (animal OR rodent OR rat OR mouse OR hamster) AND (nasal OR nose OR buccal OR larynx OR lung OR mouth OR pharynx OR sinus OR trachea) AND (cancer OR dysplasia OR neoplasia OR tumor OR carcinoma OR polyp OR cytotoxicity OR neoplastic OR promoter OR pathology OR toxicity) NOT (formalin test OR formaldehyde fixation OR formalin fixed OR formaldehyde fixed OR formalin-induced OR formaldehyde-induced)

Table A-97. Inclusion and exclusion criteria for studies of nasal cancers in animals

	Included	Excluded
Population	• Experimental animals	• Not animal studies
Exposure	• Exposure to formaldehyde for an exposure duration longer than short term	• Not related to formaldehyde (e.g., other chemicals) • Mixture studies • Short study duration
Comparison	• Inclusion of a comparison group (e.g., pre- or postexposure; no exposure; lower formaldehyde exposure level)	•
Outcome	• Endpoint evaluation included nasal cancers	• Exposure or dosimetry studies • Related to formaldehyde use in methodology • Endpoint not nasal cancer
Other	• Original primary research article	• Not a unique, primary research article, including reviews, reports, commentaries, meeting abstracts, duplicates, or untranslated foreign language studies (these were determined to be off topic or unlikely to have a significant impact based on review of title, abstract, and/or figures). • Related to policy or current practice (e.g., risk assessment/management approaches or modeling studies)

Identification of additional articles

The reference lists of the review articles identified through the process described above were manually screened (based on the criteria used for full text screening presented in Figure A-38) for relevant articles (aka "snowball searching"). These were then compared against the 229 articles identified from the computerized searches. No additional (0) relevant articles were identified.

Manual screening for relevance: Title/Abstract/Full Text

The primary research articles identified were screened within an EndNote library for relevance; title, abstract, and full text were assessed simultaneously. The number of articles excluded within each category described in Table A-97 is shown in Figure A-38.

Overall, 19 articles were identified as relevant and are cited in the animal nasal cancer section of the Formaldehyde Toxicological Review (see Appendix B.4 for individual study evaluations).

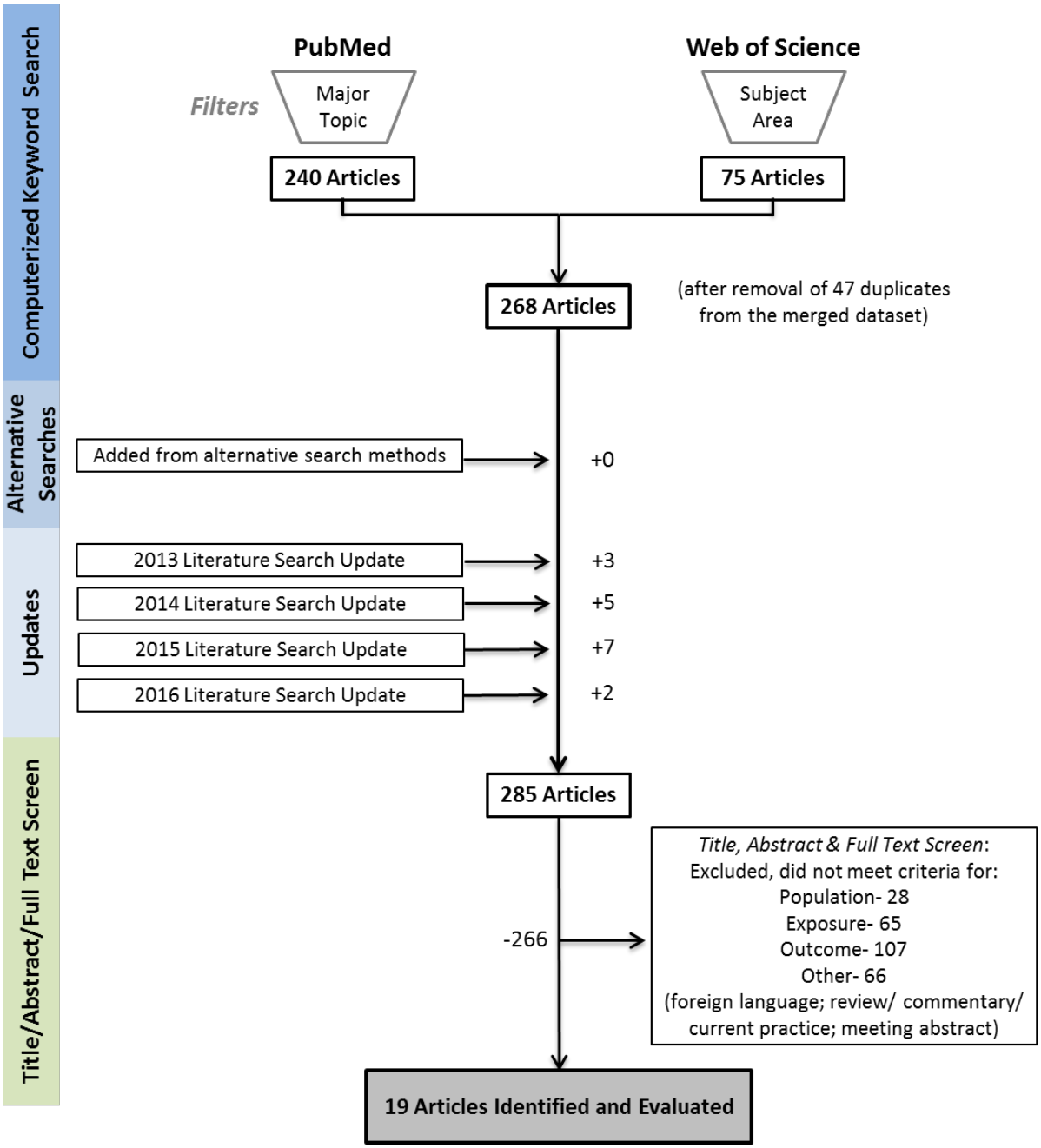


Figure A-38. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and upper respiratory tract (nasal) cancers in animals.

Lymphohematopoietic cancers (leukemia/lymphoma) in animals

A systematic evaluation of the literature database on studies examining the potential for lymphohematopoietic cancers following formaldehyde exposure was conducted through September 2016. This search strategy is summarized in Figure A-39; the search strings used in specific databases are shown in Table A-98 with additional details of the process described below, and the criteria used for inclusion and exclusion of studies during screening described in Table A-99.

Table A-98. Summary of search terms for lymphohematopoietic cancers in animals

Database, search date	Terms
PubMed 04/15/2013 No date restriction	Formaldehyde [majr] AND (leukemia OR lymphoma OR hemolymphoreticular) AND (animal OR rodent OR monkey) NOT (formalin test OR formaldehyde fixation OR formalin fixed OR formaldehyde fixed OR formalin-induced OR formaldehyde-induced)
Web of Science 03/08/2013 No date restriction Lemmatization "off"	Formaldehyde (title) AND (leukemia OR lymphoma OR hemolymphoreticular) AND (animal OR rodent OR monkey) NOT (formalin test OR formaldehyde fixation OR formalin fixed OR formaldehyde fixed OR formalin-induced OR formaldehyde-induced) (topic)

Table A-99. Inclusion and exclusion criteria for studies of LHP cancers in animals

	Included	Excluded
Population	• Experimental animals	• Not animal studies
Exposure	• Exposure to formaldehyde	• Not related to formaldehyde (e.g., other chemicals)
Comparison	• Inclusion of a comparison group (e.g., pre- or postexposure; no exposure; lower formaldehyde exposure level)	•
Outcome	• Endpoint evaluation included LHP cancers	• Exposure or dosimetry studies • Related to formaldehyde use in methodology • Endpoint unrelated to LHP cancer
Other	• Original primary research article	• Not a unique, primary research article, including reviews, reports, commentaries, meeting abstracts, duplicates, or untranslated foreign language studies (these were determined to be off topic or unlikely to have a significant impact based on review of title, abstract, and/or figures).

Identification of additional articles

The reference lists of the review articles identified through the process described above were manually screened (based on the criteria used for full text screening presented in Figure A-39) for relevant articles (aka "snowball searching"). These were then compared against the articles identified from the computerized searches to identify additional relevant articles.

Manual screening for relevance: title/abstract/full text

The primary research articles identified from database searches and evaluation of reference lists in reviews, were screened within an Endnote library for relevance; given the relatively small size of the database, title, abstract, and full text were assessed simultaneously. The number of articles excluded within each category described in Table A-99 is shown in Figure A-39.

Overall, 4 articles were identified as relevant and are cited in the animal lymphohematopoietic cancer section of the Formaldehyde Toxicological Review (see Appendix A.5.9 for individual study evaluation)

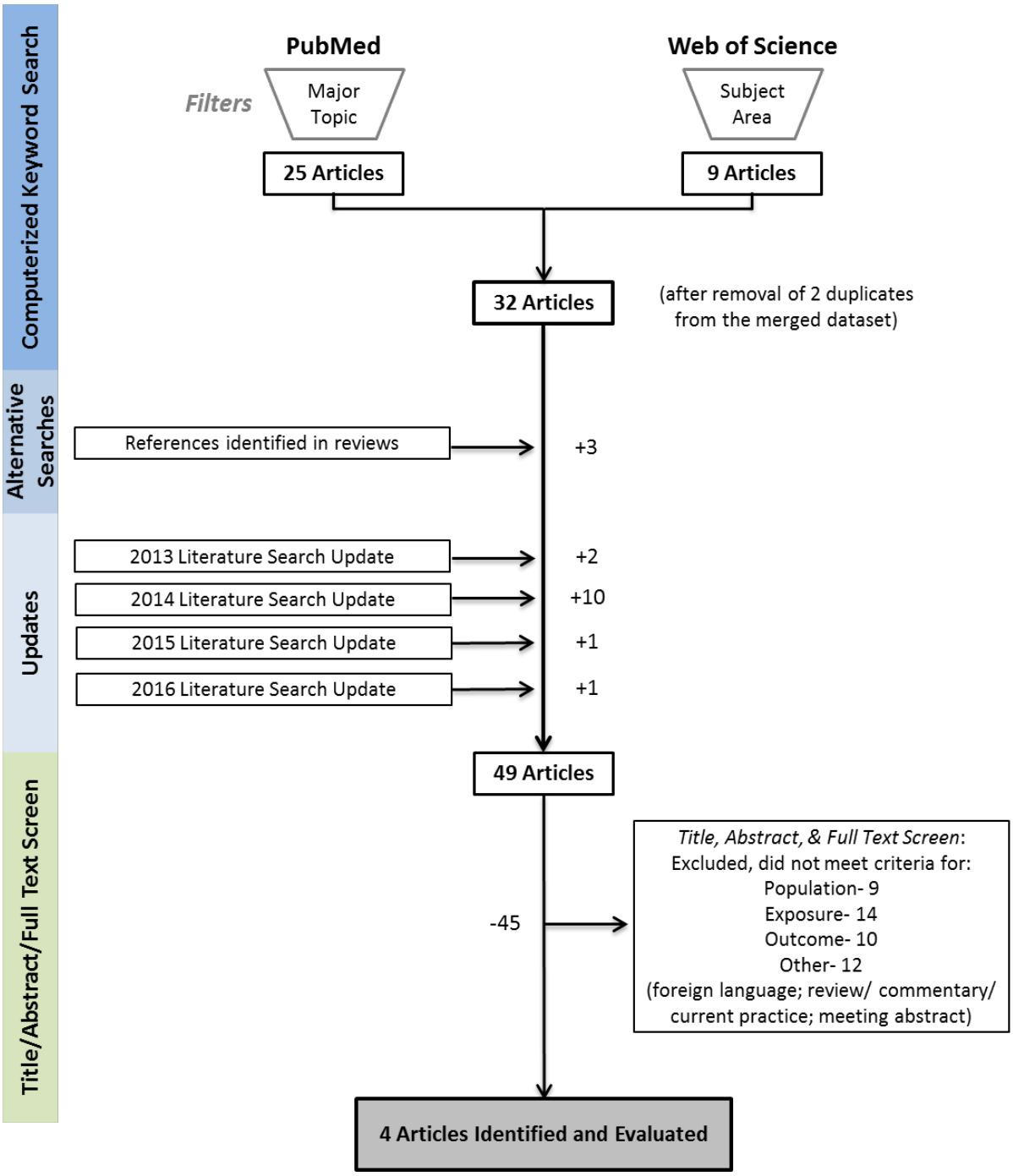


Figure A-39. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and lymphohematopoietic (LHP) cancers in animals.

Study Evaluations

Studies in Humans

The studies identified for inclusion in the review were evaluated using a systematic approach to identify strengths and limitations, and to rate the overall confidence in the results. The accompanying tables in this section document the evaluation of these studies (cohort studies, and nested case-control studies within occupational cohorts, in Table A-105, and case-control studies in Table A-106). Studies are arranged alphabetically by author within each table.

The focus of EPA's examination is on several specific types of upper respiratory tract (URT) and lymphohematopoietic (LHP) cancer. The evaluation of LHP cancers includes four different subtypes: myeloid leukemia (including monocytic leukemia), lymphatic leukemia, multiple myeloma, and Hodgkin lymphoma. Among upper respiratory cancers, four different types are reviewed: sinonasal (SNC), nasopharyngeal cancer (NPC), oro/hypopharyngeal cancer (OHPC), and laryngeal cancer.

Evaluation of Observational Epidemiology Studies of Cancer

The epidemiology studies examined occupational exposure to formaldehyde either in specific work settings (e.g., cohort studies) or in case-control studies. The considerations with respect to design, exposure assessment, outcome assessment, confounding and analysis differ for these different types of studies, and are discussed in more detail below.

Each study identified by the literature search as potentially relevant to inform the causal evaluation of whether formaldehyde exposure causes cancer was then evaluated and classified for the study's ability to inform a hazard conclusion for a particular cancer outcome. Study evaluation encompasses interpretations regarding a variety of methodological features (e.g., study design, exposure measurement details, study execution, data analysis). Developing an outcome-specific study evaluation for each cancer outcome encompasses two concepts: minimization or control of bias (internal validity), and sensitivity/appropriateness (the ability of the study to detect a true effect). The purpose of this step is not to eliminate studies, but rather to evaluate studies with respect to potential methodological considerations that could affect the interpretation of or confidence in the results.

1) Consideration of participant selection and comparability

- Whether there is evidence of selection into or out of the study (or analysis sample) that was jointly related to exposure and to outcome.

  For cohort studies, EPA considered the extent of follow-up, and the likelihood that completeness of follow-up was related to exposure level. Most of the cohort studies examining mortality data reported high rates of follow-up with respect to ascertainment of vital status and ascertainment of cause of death (90–95% or higher); in some cases, the latter figure (i.e., percentage of decedents with death certificates) was not provided

by the study authors. Two studies were able to obtain only 79% (Hayes et al., 1990) or 75% (Walrath and Fraumeni, 1984) of the identified death certificates but as both studies were of embalmers who were all considered to have been exposed to formaldehyde, the absence of data (missingness) was considered to have been random.

For case-control studies, controls are optimally selected to represent the population from which the cases were drawn (e.g., similar geographic area, socioeconomic status, and time period). A variety of methods were used in the identified studies, including random digit dialing and use of population registries. The interest and motivation to participate is generally higher for cases than for controls, particularly in population-based settings. A low participation rate of either or both groups does not in itself indicate the occurrence of selection bias; a biased risk estimate is produced if exposure and disease are jointly related to participation rates, but not if either is independent of participation rates. For example, a bias is not necessarily produced if cases are more likely to participate than controls; a bias can be produced, however, if cases with high exposure are more likely to participate than cases with low exposure. Most of the case-control studies were conducted using incident (or recently diagnosed) cases, with participation rates ranging from approximately 75% to 99%. Participation among population-based controls generally ranged from 75% to 85%, with higher rates seen in some studies using with hospital-based. Differences in participation rates between case and controls potentially related to exposure were considered to be more prone to be biased [Armstrong, 2000, 2452550}. Certain studies used cases' next of kin to ascertain the cases' occupational history from which the individual's exposure to formaldehyde was derived. The difference in methods for ascertaining exposure histories thus differs between deceased cases and the controls and creates a potential for selection bias (e.g., (Yang et al., 2005; Vaughan, 1989; Vaughan et al., 1986a, b).

- An uncommon issue related to potential selection bias was the "healthy worker effect" in cohort studies where a working population compared to that of the general public—a bias which can result in underestimates of any adverse effect of exposure. While this phenomenon is generally considered to be a stronger influence in evaluation of cardiovascular health endpoints, there is evidence that there can be a strong healthy worker effect in studies of cancer endpoints (Sont et al., 2001). In cohort studies, the potential for selection bias due to the healthy worker effect was assessed by examination of the all-cause cancer effect estimates; studies with estimates <90% of expected were judged to be potentially biased towards lower overall cancer occurrence and lower levels of cases detection resulting in underestimates of any true effect. Severe underestimates of <80% of expected cases were noted as well (e.g., e.g., Wesseling et al., 1996; Hall et al., 1991; Matanoski, 1989; Robinson et al., 1987; Stroup et al., 1986; Harrington and Oakes, 1984; Levine et al., 1984b).

- For some cancers, the reliance of cohort studies on death certificates to detect cancers with relatively high survival may have underestimated the actual incidence of those cancers, especially when the follow-up time may have been insufficient to capture all cancers that may have been related to exposure. The potential for bias may depend upon the specific survival rates for each cancer. Five-year survival rates vary among the selected cancers (see Table A-100), from 86% for Hodgkin lymphoma (HL) to less than 50% for multiple myeloma (MM), myeloid leukemia (ML), and oro/hypopharyngeal cancer. EPA considered the likelihood of underreporting of incident cases to be higher for mortality-based studies of HL and LL which may result in undercounting of incident cases and underestimates of effect

estimates compared to general populations (e.g., Mayr et al., 2010; Hansen and Olsen, 1995; Hansen et al., 1994; Hayes et al., 1990; Solet et al., 1989).

Table A-100. Lymphohematopoietic and upper respiratory cancers: age-Adjusted SEER incidence and U.S. death rates and 5-year relative survival by primary cancer site[a]

Cancer Site	Incidence Rate (per 100,000) 2008–2012	Expected Cases[b] 2014	Mortality Rate (per 100,000)[c] 2008–2012	Expected Deaths[b] 2014	5-Year Survival (%) 2005–2011
Lymphohematopoietic Cancers					
Hodgkin lymphoma (HL)	2.7	8,336	0.4	1,235	85.9
Multiple myeloma (MM)	6.3	19,451	3.3	10,189	46.6
Lymphatic Leukemia (LL)	6.6	20,377	1.9	5,866	77.6
Acute lymphatic leukemia (ALL)	1.7	5,249	0.4	1,235	67.5
Chronic lymphatic leukemia (CLL)	4.5	13,894	1.4	4,322	81.7
Other	0.4	1,235	0.1	309	80.6
Myeloid & monocytic leukemia (ML)	6.1	18,833	3.4	10,497	37.5
Acute myeloid leukemia (AML)	4.0	12,350	2.8	8,645	25.9
Chronic myeloid leukemia (CML)	1.7	5,249	0.3	926	63.2
Acute monocytic	0.2	617	0.0	0	23.5
Other	0.2	617	0.2	617	33.2
Upper Respiratory Tract Cancers					
Nose, nasal, & middle ear[e]	0.7	2,161	0.1	309	55.3
Nasopharynx	0.6	1,852	0.2	617	59.6
Oropharynx	0.4	1,235	0.2	617	41.7
Hypopharynx	0.6	1,852	0.1	309	32.2
Larynx	3.2	9,880	1.1	3,396	60.6

[a]Incidence rates and 5-year survival from Surveillance, Epidemiology, and End Results (SEER), 18 areas. Results. [http://seer.cancer.gov/csr/1975_2012/results_merged/topic_survival.pdf], last accessed August 14, 2015.
[b]EPA calculated the expected number of cases based on incidence rates applied to U.S. census population estimate for 2014 of 308,745,538 (http://www.census.gov/search-results.html?q=2014+population&page=1&stateGeo=none&searchtype=web).
[c]U.S. Mortality Files, National Center for Health Statistics, Centers for Disease Control and Prevention
[d]SEER 18 areas. Based on follow-up of patients into 2012.
[e]SEER does not publish specific data on sinonasal cancer which would be included in the published category labeled "Nose, nasal & middle ear."

2) The reliance of case-control studies on prevalent cases rather than incident cases.

In order to accrue a sufficiently large population of rare cancer cases, some studies may include cases which have been detected over a long period of time and thus include many prevalent cases at the time of analysis. Restriction to only living cases may lead to over-representation of

cancer survivors or, if next of kin are used to provide proxy information on cases, the quality of that data may then differ between cases and controls which can be a concern if differences may be related to exposure. Hence, EPA considers that there is some risk of selection bias in studies examining prevalent cases (e.g., Mayr et al., 2010; Pesch et al., 2008; Yang et al., 2005; Armstrong et al., 2000; Vaughan, 1989; Vaughan et al., 1986a, b).

3) Evaluation of exposure assessment

At a minimum, exposure to formaldehyde may be inferred based on the specific occupations (e.g., carpenter, embalmer, pathologist) or industry (e.g., production or use of formaldehyde resins, wood-products, paper, textiles, foundries). Independent testing of various workplaces may provide approximate exposure measurements and ranges for inferred exposures. Details in each study may reveal the extent of exposure within occupational groups or at the individual-level based on job histories. Some studies may have documented formaldehyde exposures using exposure monitors or quantified the absolute or relative exposure for different tasks, which may be matched to individual occupational patterns using "job exposure matrices" or JEMs. The quality of the exposure measure is evaluated with respect to the accuracy of the measures and their related potential for exposure measurement error which can lead to "information bias." The overwhelming majority of information bias in epidemiologic studies of formaldehyde stems from the use of occupational records to gauge exposures with some degree of exposure misclassification or exposure measurement error considered to be commonplace.

A primary consideration in the evaluation of these studies is the ability of the exposure assessment to reliability distinguish among levels of exposure within the study population, or between the study population and the referent population. A large variety of occupations are included within the studies; some represent work settings with a high likelihood of exposure to high levels of formaldehyde, and some represent work settings with variable exposures and in which the proportion of people exposed is quite small. In the latter case, the potential effect of formaldehyde would be "diluted" within the larger study population, limiting the sensitivity or informative nature of the study. EPA categorized the exposure assessment methods of the identified studies into four groups (A through D), reflecting greater or lesser degree of reliability and sensitivity of the measures (see Table A-101). Outcome-specific association based on Group A exposures were consider without appreciable information bias due to exposure measurement error while those based on Groups B–D were considered to be somewhat biased towards the null.

Table A-101. Categorization of exposure assessment methods by study design.

Group	Cohort (and nested case-control within cohort) studies	Case-control and cancer registry-based studies
A	Industrial settings with extensive industrial hygiene data used to determine levels of exposure (and	Detailed lifetime job history, more extensive than industry and occupation codes, including information about specific tasks and setting,

Group	Cohort (and nested case-control within cohort) studies	Case-control and cancer registry-based studies
	variability within a worksite); job exposure matrix takes into account variability by time and job/task. • (Beane Freeman et al., 2013; Beane Freeman et al., 2009) Highly exposed professions (embalmers) with comparison to general population, or with measures capturing variability within the cohort (Hauptmann et al., 2009) • (Hayes et al., 1990) • (Levine et al., 1984b) • (Meyers et al., 2013) • (Stroup et al., 1986) • (Walrath and Fraumeni, 1983) • (Walrath and Fraumeni, 1984)	combined with job exposure matrix that takes into account variability by time, setting, and job/task. Also includes some kind of validation study or congruence of ratings based on different exposure ascertainment measures to be equivalent to Group A cohort studies with extensive industrial hygiene data. • (none identified)
B	Industrial settings with more limited industrial hygiene data • (Andjelkovich et al., 1995) • (Coggon et al., 2014; Coggon et al., 2003) • (Edling et al., 1987b) • (Fryzek et al., 2005) • (Marsh et al., 2007; Marsh et al., 2002) • (Ott et al., 1989) Exposed professions (e.g., pathologists) with comparison to general population, but that do not have measures capturing variability within the cohort • (Bertazzi et al., 1989) • (Hall et al., 1991) • (Harrington and Oakes, 1984) • (Li et al., 2006) • (Matanoski, 1989)	Detailed lifetime job history, more extensive than industry and occupation codes, including information about specific tasks and setting, combined with job exposure matrix that takes into account variability by time, setting, and job/task. • (Armstrong et al., 2000) • (D'Errico et al., 2009) • (Gérin et al., 1989) • (Gustavsson et al., 1998) • (Hildesheim et al., 2001) • (Pesch et al., 2008) • (Vaughan et al., 2000)
C	Industrial settings that are only able to use duration as a way to distinguish variability in exposure • (Band et al., 1997) • (Dell and Teta, 1995) • Self-report of exposure • (Boffetta et al., 1989) • (Saberi Hosnijeh et al., 2013) • (Stellman et al., 1998)	Lifetime job history coding based only on industry and occupation; more detailed information about specific tasks and setting not included in assessment of exposure potential (or, information on what was collected was not provided) • (Blair et al., 2001) • (Laforest et al., 2000) • (Luce et al., 2002) • (Olsen et al., 1984) • (Olsen and Asnaes, 1986b) • (Roush et al., 1987)

Group	Cohort (and nested case-control within cohort) studies	Case-control and cancer registry-based studies
		• (Shangina et al., 2006) • (West et al., 1993) • (Wortley et al., 1992) • (Yu et al., 2004) • Self-report of exposure • (Mayr et al., 2010) Lifetime job history, including tasks/exposure information, but analysis conducted only for job categories rather than for an exposure category • (Teschke et al., 1997)
D	Industrial settings that do not include data to distinguish variability in exposure (e.g., wood workers, with no information on which workers were exposed to formaldehyde; textile workers with no formaldehyde exposure measures), or that include few people classified as exposed • (Hansen et al., 1994) pharmaceuticals • (Hansen and Olsen, 1995) plant used 1kg/person/yr • (Jakobsson et al., 1997) grinding stainless steel • (Malker et al., 1990) fiberboard plants • (Siew et al., 2012) any occupational exposure • (Solet et al., 1989) pulp and paper mills • (Robinson et al., 1987) plywood mill workers • Wesseling, 1996, 1986612} banana plant workers Methods of exposure assessment rated as higher quality but downgraded due to methods used by study authors which were likely to induce bias. • (Checkoway et al., 2015)	Job history limited to information on a single job (e.g., based on tax record, death certificate, medical record, census data) • (Heineman et al., 1992) • (Pottern et al., 1992) • (Talibov et al., 2014) High proportion (> 40%) of next-of-kin interviews • (Vaughan, 1989; Vaughan et al., 1986a, b) • (Yang et al., 2005) Methods of exposure assessment rated as higher quality but downgraded due to validation by study authors. • (Berrino et al., 2003)

Additional exposure measurement error may arise in circumstances when the time period of exposure assessment is not well aligned with the time period when formaldehyde exposure could induce carcinogenesis that develops to a detectable stage (incident cancer) or result in death from a specific caner. Epidemiology studies regularly explore the analytic impact of different lengths of 'latency periods' which may exclude from the analyses the formaldehyde exposure most proximal to each individual's cancer incidence or cancer mortality. For analyses of the exposure-

related risks of solid tumors, it is commonplace evaluate latency periods of 10, 15, or 20 years by present results stratified by time since first exposure or to exclude (or in the parlance of epidemiology, to "lag") exposures in the 10, 15, or 20 years immediately prior to death from the analyses so as to more accurately (potentially) describe what may be the more biologically relevant window of exposure in time that could have caused carcinogenesis (sometimes called the etiologically relevant time period). Analyses which do not evaluate latency, may be inducing exposure measurement error by including irrelevant exposure and were considered to be somewhat biased towards the null.

An understanding of the effects of exposure measurement error on the results from epidemiologic analyses is important as it enables the reviewer to place these possible exposure measurement errors in context. The effect of exposure measurement error on estimates of the risk of cancer mortality potentially attributable to formaldehyde exposure depends upon the degree to which that error itself may be related to the likelihood of the outcome of interest. Exposure measurement error that is similar among both workers who died of a specific cancer, and those who did not die of that cancer, is termed nondifferential exposure measurement error. Exposure measurement error that is associated with the outcome (error that is differential with respect to disease status) can cause bias in an effect estimate towards or away from the null, while nondifferential exposure error typically results in bias towards the null (Rothman and Greenland, 1998).

4) Outcome measure

The diagnosis of cancers in epidemiologic studies has historically been ascertained from death certificates according to the version of the International Classification of Diseases (ICD) in effect at the time of study subjects' deaths [i.e., ICD-8 and ICD-9: (WHO, 1977, 1967)]. The most specific classification of diagnoses that is commonly reported across the epidemiologic literature has been based on the first three digits of the ICD code (i.e., Myeloid Leukemia ICD-8/9: 205) without further differentiation (i.e., Acute Myeloid Leukemia ICD-8/9: 205.0)—although some studies have reported results at finer levels. In the evaluation of the epidemiologic evidence for upper respiratory cancers, four different types are reviewed: sinonasal cancer, nasopharyngeal cancer, oro/hypopharyngeal cancer, and laryngeal cancer. In the evaluation of the epidemiologic evidence for LHP cancers, four different subtypes are reviewed: myeloid leukemia (including monocytic leukemia), lymphatic leukemia, multiple myeloma, and Hodgkin lymphoma. In restricting the causal evaluation of LHP cancers to these four specific subtypes, another category of LHP cancer originating from white blood cells, which includes all lymphoma not classified as Hodgkin was not evaluated.

In the review of study quality for cancer studies, the outcome measure was generally considered to be accurate as the source of this information was typically from death certificates, cancer registries, or hospitals. Some studies did provide additional information on histological

typing but the majority did not. Histological type can be informative in understanding the epidemiologic evidence but the lack of such information was not judged as a major study limitation. While it is true that death certificates and other administrative records can occasionally contain errors, the impact of misclassification of outcome on epidemiologic results is to reduce precisions in effect estimates and not to induce bias.

5) Consideration of likely confounding

EPA evaluated the potential for confounding based on exposures to identified risk factors for specific, or related, cancers, whether those exposures were found to be risk factors in the specific study and whether there was a known or likely correlation between those exposures and formaldehyde. Information on the presence of potential confounders in a particular study was gleaned from the study itself or from information from outside the study (e.g., information on exposure levels from other sources).

Risk factors for LHP cancers include pharmaceuticals (chemotherapeutic drugs), biological agents (e.g., viruses), radiation, and chemical exposures (Cogliano et al., 2011). The primary agents of interest that were considered in the study quality review are the potential occupational and environmental co-exposures that may be associated with formaldehyde exposure as well as LHP cancers. Chemotherapeutic drug exposures were not expected to be correlated with formaldehyde exposures during the etiologically relevant time period for potentially formaldehyde-related carcinogenesis and were not considered as potential confounders. Similarly, viral exposures and radiation exposures also were not expected to be correlated with formaldehyde exposures except, possibly, among embalmers and pathologists who may be co-exposed by deceased persons who had viral infections or had implanted radiation devices used in chemotherapy. Each of the chemical and occupational exposures that were reported to be associated with risks of LHP cancers (i.e., benzene, 1,3-butadiene, 2,3,7,8-tetrachlorodibenzo-para-dioxin, ethylene oxide, magnetic fields, paint, petroleum refining, polychlorophenols, radioisotopes and fission decay products, styrene, tetrachloroethylene, tobacco smoking, trichloroethylene; (Cogliano et al., 2011) was examined in the study quality review and evaluated as a potential confounder of any association between formaldehyde and specific LHP cancers.

Risk factor for URT cancers include biological agents (e.g., viruses), radiation, and chemical exposures (Cogliano et al., 2011). Viral exposures and radiation exposures also were not expected to be correlated with formaldehyde exposures except, possibly, among embalmers and pathologists who may be co-exposed by deceased persons who had viral infections or had implanted radiation devices used in chemotherapy. Each of the chemical and occupational exposures which were reported to be associated with risks of URT cancers (i.e., acid mists, asbestos, chromium VI, isopropyl alcohol production, leather dust, nickel compounds, radioisotopes and fission decay products, rubber production, textile manufacturing, tobacco smoking, wood dust; (Cogliano et al.,

2011) was examined in the study quality review and evaluated as a potential confounder of any association between formaldehyde and specific URT cancers.

The specific chemical and occupational exposures, listed above, which were reported to be associated with LHP or URT cancers are **bolded** in the lists of co-exposures in each study in the Exposure Measure column of the study quality tables. This identifies any important co-exposures which are then evaluated for their potential correlation with formaldehyde exposure to identify potential confounders.

6) Analysis and results (estimate and variability)

Analyses should be appropriate with respect to study design. When analytic methods are not matched to the study design, the expected impact on the results was evaluated. For cancer endpoints, results that examined the effects of including various latency periods using lagged exposure of strata of time since first exposure allow for the focus of results on different etiological windows of time that may be more biologically relevant. Studies that did not report results looking at different latencies may be vulnerable to additional exposure measurement error as they evaluate the effects of formaldehyde exposures during times that may not have any causal effects such as in the years immediately preceding death.

7) Study sensitivity

Studies with small cases counts may have little statistical power to detect divergences from the null but are not necessarily expected to be biased and no study is excluded solely on the basis of cases counts as this methodology would excluded any study which saw no effect of exposure. Therefore, cohort studies with extensive follow-up which reported outcome-specific results on a number of different cancers, including very rare cancers such as NPC and SNC, are evaluated even when few or even no cases were observed, if information on the expected number of cases in the study population was provided so that confidence intervals could be presented to show the statistical uncertainty in the associated effect estimated. For example, Coggon et al. (2014) followed the mortality of 14,008 workers and yet expected only 1.7 deaths from nasopharyngeal cancer in the exposed workers and observed just one resulting in an unstable estimated RR=0.38 (95% CI: 0.02–1.90). Meyers et al. (2013) followed the mortality of 11,043 workers and expected only 1.33 deaths from nasopharyngeal cancer and did not observe any deaths, resulting in a SMR=0 (95% CI: 0–2.77). In general, cohort studies should have a sufficiently long follow-up period for any exposure-related cancer cases to develop and be detected and ideally, allow for analyses of potential cancer latency. Outcome-specific effect estimates from cohort studies with short follow-up could be uninformative depending on the size of the study population and the baseline frequency of the cancer.

Outcome-specific evaluation of confidence in the precise effect estimate of an association

An outcome-specific evaluations classified with **High** confidence in the precise effect estimate is expected to be without appreciable bias and thus represents an accurate estimate of any reported association between formaldehyde exposures and the risks of cancer. These evaluations are expected to have methodological features sufficiently sensitive to provide an adequate basis for interpreting null or weak results as evidence of no or weak risk of cancer. Table A-102 identifies the outcome-specific evaluations were classified with High confidence.

Table A-102. Outcome-specific effect estimates classified with High confidence

Reference	Outcome-specific effect estimates	Confidence classification
(Beane Freeman et al., 2009)	Hodgkin Lymphoma	High
(Beane Freeman et al., 2009)	Larygeal cancer	High
(Beane Freeman et al., 2013)	Lymphocitic leukemia	High
(Beane Freeman et al., 2009)	Multiple myeloma	High
(Beane Freeman et al., 2009)	Myeloid leukemia	High
(Beane Freeman et al., 2013)	Nasopharyngeal cancer	High
(Hauptmann et al., 2009)	Multiple myeloma	High
(Hauptmann et al., 2009)	Myeloid leukemia	High
(Meyers et al., 2013)	Multiple myeloma	High
(Meyers et al., 2013)	Myeloid leukemia	High

An outcome-specific evaluation classified with **Medium** confidence in the precise effect estimate may have some potential for residual bias, but the direction of the observed effect is unaffected and the magnitude of any expected biases are limited. Thus, the observed effect estimates represent a reasonable estimate of the association between formaldehyde exposures and the risk of cancer, and are expected to be sufficiently sensitive to provide an adequate basis for interpreting null or weak results as evidence of no or weak risk of cancer. Table A-103 identifies the outcome-specific evaluations were classified with Medium confidence.

Table A-103. Outcome-specific effect estimates classified with Medium confidence

Reference	Outcome-specific effect estimates	Confidence classification
(Beane Freeman et al., 2009)	Hodgkin lymphoma	Medium
(Beane Freeman et al., 2009)	Lymphocytic leukemia	Medium
(Beane Freeman et al., 2013)	Sinonasal cancer	Medium
(Coggon et al., 2014)	Myeloid leukemia	Medium
(Coggon et al., 2014)	Laryngeal cancer	Medium
(Coggon et al., 2014)	Oro/hypopharyngeal cancer	Medium
(Gérin et al., 1989)	Hodgkin lymphoma	Medium
(Hayes et al., 1990)	Multiple myeloma	Medium

Reference	Outcome-specific effect estimates	Confidence classification
(Hayes et al., 1990)	Myeloid leukemia	Medium
(Hauptmann et al., 2009)	Lymphatic leukemia	Medium
(Hildesheim et al., 2001)	Nasopharyngeal cancer	Medium
(Meyers et al., 2013)	Oro/hypopharyngeal cancer	Medium
(Walrath and Fraumeni, 1983)	Myeloid leukemia	Medium
(Walrath and Fraumeni, 1984)	Myeloid leukemia	Medium
(Laforest et al., 2000)	Oro/hypopharyngeal cancer	Medium
(Luce et al., 2002)	Sinonasal cancer	Medium
(Olsen and Asnaes, 1986b)	Sinonasal cancer	Medium
(Olsen et al., 1984)	Nasopharyngeal cancer	Medium
(Roush et al., 1987)	Nasopharyngeal cancer	Medium
(Roush et al., 1987)	Sinonasal cancer	Medium
(Vaughan et al., 2000)	Nasopharyngeal cancer	Medium
(West et al., 1993)	Nasopharyngeal cancer	Medium

An outcome-specific evaluation classified with **Low** confidence in the precise effect estimate is likely to have some residual bias or may lack sensitivity to provide an adequate basis for interpreting null or weak results as evidence of no or weak risk of cancer. For example, an outcome-specific effect estimate based on fewer than five observed or expected cases of a particular cancer would be classified with Low confidence based on a lack of sensitivity, even if there were no appreciable biases. Another study classified with Low confidence might have relied on exposure assessment methodologies that were unbiased, but nonspecific in nature so as to yield effect estimates that were likely biased towards the null, and thus, underestimated any true effect. Similarly, the lack of consideration of latency is a limitation as it may cause measurement error in improperly including exposure of little biological relevance to cancer occurrence. Concern about the potential for confounding is a limitation when a co-exposure is a known cause of a particular cancer endpoint and may be correlated with formaldehyde exposure is a study. Selection bias may be a limitation when survival rates are long as incidence cases may not be readily detected using mortality statistics. In general, outcome-specific effect estimates that underestimate any true effect may still inform a hazard conclusion. However, outcome-specific effect estimates that overestimate any true effect cannot inform a hazard conclusion and are considered to be uninformative as are outcome-specific effect estimates, which suffer from strong bias or a complex mixture of biases. Tables A-105 and A-106 identify the outcome-specific evaluations that were classified with Low confidence.

Exclusion of studies based judged to be uninformative for the evaluation of causation

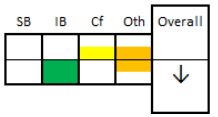
In rare circumstances, studies initially judged to be potentially informative were further evaluated and found to be uninformative. For example, studies of specific LHP subtypes, which mention formaldehyde or study the health of workers in an industry expected to be exposed to

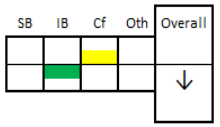
formaldehyde but details of the study reveal only extremely limited exposure (Armstrong et al., 2000; Dell and Teta, 1995) or virtually none at all (Li et al., 2006). Two outcome-specific associations were judged to be uninformative due, in part, to potential manifestations of the healthy worker effect with standardized mortality ratio for "all cancers" more than 30% below expected values (SMR<0.7:Hall et al., 1991; Harrington and Oakes, 1984). Another reason was that a study had co-exposures that are likely to have been highly correlated with formaldehyde and were known risk factors for LHP cancers and the independent effect of formaldehyde cannot be inferred (e.g., D'Errico et al., 2009; Fryzek et al., 2005). Studies with co-exposures to known risk factors for LHP cancers that are not likely to be highly correlated for formaldehyde or were not risk factor for the specific LHP subtype in question are included and the potential for confounding is noted for evaluation in the causal synthesis. Table A-104 identifies the outcome-specific evaluations were classified as uninformative.

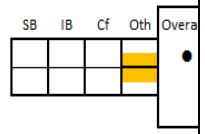
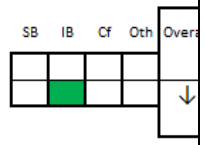
Table A-104. Outcome-specific effect estimates classified as uninformative

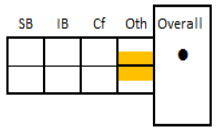
Reference	Outcome-specific effect estimates	Confidence classification	Critical limitation(s)
(Armstrong et al., 2000)	Nasopharyngeal cancer	Not informative	Multiple
(Berrino et al., 2003)	Laryngeal cancer	Not informative	Confounding
(D'Errico et al., 2009)	Sinonasal cancer	Not informative	Confounding
(Dell and Teta, 1995)	Nasopharyngeal cancer	Not informative	Sensitivity (minimal exposure)
(Fryzek et al., 2005)	Hodgkin lymphoma	Not informative	Confounding
(Fryzek et al., 2005)	Multiple myeloma	Not informative	Confounding
(Hall et al., 1991)	Hodgkin lymphoma	Not informative	Selection bias (healthy worker effect)
(Hansen et al., 1994)	Hodgkin lymphoma	Not informative	Information bias (minimal exposure)
(Hansen et al., 1994)	Laryngeal cancer	Not informative	Information bias (minimal exposure)
(Hansen et al., 1994)	Multiple myeloma	Not informative	Information bias (minimal exposure)
(Harrington and Oakes, 1984)	Sinonasal cancer	Not informative	Selection bias (healthy worker effect)
(Li et al., 2006)	Nasopharyngeal cancer	Not informative	Sensitivity (minimal exposure)
(Li et al., 2006)	Sinonasal cancer	Not informative	Sensitivity (minimal exposure)
(Matanoski, 1989)	Hodgkin lymphoma	Not informative	Selection bias and Information bias
(Mayr et al., 2010)	Sinonasal cancer	Not informative	Confounding
(Solet et al., 1989)	Hodgkin lymphoma	Not informative	Multiple
(Wesseling et al., 1996)	Hodgkin lymphoma	Not informative	Multiple
(Wesseling et al., 1996)	Multiple myeloma	Not informative	Multiple

Table A-105. Evaluation of occupational cohort studies of formaldehyde and cancers of the URT (NPC, SN, OHPC) and LHP (HL, MM, LL, ML)

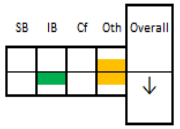
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
Andjelkovich et al. (1995) United States Cohort study of iron foundry workers working during 1960–1987 with follow-up through 1989.	3,929 male workers exposed to formaldehyde ≥ 6 mos. Loss to follow-up 1.3% (1.5% of 2,032 unexposed workers). Median follow-up ≈15 yrs. Average follow-up ≈20.77 yrs. All cancer SMR = 0.99.	Individual-level exposure (Yes/No), questionnaire based on industrial hygienist review of detailed work histories; assignments based on job title and industrial hygiene data and information on tasks and plants. Exposure assessment blinded to outcome. Co-exposed to silica. Possibly co-exposed to polycyclic aromatic hydrocarbons, **nickel**, and **chromium**.	Mortality: underlying cause of death based on ICD-8 (Social Security Administration Pension Benefit Information, and National Death Index). HL: ICD 201. Higher survival rates for HL could undercount incident cases, but median follow-up is more than 15 yrs.	Controlled for sex, age, race, and calendar-year specific mortality rates. Nickel and chromium are associated with URT cancers and would likely be positively correlated with formaldehyde exposure. Potential for confounding is unknown but could have inflated the observed effect. Other co-exposures are not known risk factors for these outcomes.	Exposed vs. unexposed. SMRs (95% CI). Latency not evaluated.	HL: 1 Larynx: 3 NPC: 0 SNC: 0	 Exposure: Group B; lack of latency analysis Confounding possible for URT cancers Low power (few cases) **SUMMARY: HL, Larynx, NPC, SNC: LOW ↓** (Low sensitivity Potential biases)

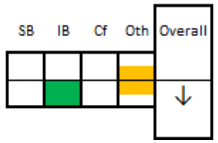
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
Band et al. (1997) Canada Cohort study of pulp and paper workers, working before 1950 with follow-up through 1982.	28,200 male workers employed at least one year by January 1950. Loss to follow-up < 6.5% for workers exposed to the sulfate process (67% of original cohort of 30,157 were exposed to the sulfate process) and loss to follow-up < 20% for workers exposed to the sulfite process. Average follow-up ≈19.42 yrs. All cancer SMP = 1.03.	Hire and termination dates and type of chemical process of pulping (sulfate vs. sulfite). Individual exposure measures not derived. As a profession, workers were likely exposed to formaldehyde. Formaldehyde is known to be an exposure for pulp and paper mill workers: job-specific exposures range from 0.2 to 1.1 ppm with peaks as high as 50 ppm (Korhonen et al., 2004). Co-exposed to arsenic, **chlorophenols**, **sulfuric acid mists**, and chloroform. Co-exposures to **dioxin** or **perchloroethylene** are also possible (Kauppinen et al., 1997).	Mortality: underlying cause of death obtained from the National Mortality Database based on ICD version in effect at time of death and standardize to ICD-9 version HL: ICD 201 MM: ICD 203. Higher survival rates for HL could undercount incident cases, but average follow-up is more than 15 yrs.	All comparisons adjusted for age and sex. Confounding not evaluated. Potential confounders for these outcomes include chlorophenols, acid mists, dioxin, and perchloroethylene and would likely be positively correlated with formaldehyde exposure. Potential for confounding is unknown but could have inflated the observed effect. Other co-exposures are not known risk factors for these outcomes.	SMRs (95% CI). Duration of exposure evaluated. Latency evaluated as time since first exposure.	HL: 7 Larynx: 12 MM: 12	 Exposure: Group C Confounding possible for LHP and URT cancers **SUMMARY:** **HL, Larynx, MM:** **LOW ↓** (Potential biases)

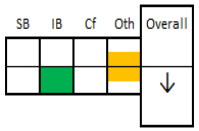
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
(Beane Freeman et al., 2013); Beane Freeman et al. (2009) United States Cohort study of workers in 10 plants using or producing formaldehyde, follow-up through 2004. Related studies: Initial 10 plant cohort follow-up through 1980 Blair et al. (1987); Blair et al. (1986). Second set of 10 plant follow-ups through 1994 Hauptmann et al. (2004a); Hauptmann et al. (2003). Reanalysis of 1 plant Marsh et al. (2007); Marsh et al. (2002). Reanalysis of Beane Freeman et al. (2009) by Checkoway et al. (2015).	25,619 workers (12% female) followed from plant start-up or first employment. Deaths were identified from the National Death Index with remainder assumed to be living. Vital status was obtained for 97.4%. Median follow-up 42 yrs. Average follow-up ≈38.96 yrs. All cancer SMR = 0.93.	Individual-level exposure estimates based on job titles, tasks, visits to plants by study industrial hygienists who took 2,000 air samples from representative jobs, and plant monitoring data from 1960 through 1980. Blinded to outcome. Median cumulative exposure was 0.6 ppm-years (range = 0.0–107.4 ppm-yrs). Co-exposed to antioxidants, **benzene**, carbon black, dyes and pigments, melamine, hexamethylenetetramine, phenols, plasticizers, urea, **wood dust**. (Beane Freeman et al., 2013) sampled cohort members and found no association between smoking	Mortality: underlying cause from death certificates, ICD-8. HL: ICD 201 MM: ICD 203 LL: ICD 204 ML: ICD 205. Larynx: ICD 161 NPC: ICD 147 SNC: ICD 160. Higher survival rates for HL and LL could undercount incident cases, but median follow-up is more than 42 years. Checkoway et al. (2015) AML: 205.0 CML: 205.1	All comparisons adjusted for calendar year, age, sex, and race. Internal analysis adjusted for pay category. For HL, MM, LL, ML: Benzene is a potential confounder but was controlled for. For NPC, SN: Wood dust is a potential confounder but was controlled for. Eleven co-exposures examined as potential confounders, but none were found to be confounders.	Internal: Poisson regression; RR (95% CI) by exposure categories (4 levels), for peak, average, cumulative exposures. Latency was evaluated. External: SMRs (95% CI). Checkoway et al. (2015) Cox PH regression; HR (95% CI) by exposure categories (4 levels collapsed to 3 by widening the ref. cat. due to small numbers). Latency was evaluated.	HL: 27 MM: 59 LL: 37 ML: 48 Larynx: 48 NPC: 11 SNC: 5 Checkoway et al. (2015)AML: 34 CML: 13	 Exposure: Group A Low power for SNC **SUMMARY: SNC: MEDIUM** (Low sensitivity) **HL, Larynx, LL, ML, MM, NPC: HIGH** Checkoway et al. (2015)  Exposure Group A from from Beane Freeman et al. (2009) downgraded to Group D based on authors' decision to reclassify all

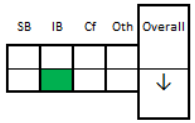
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
		and formaldehyde. Blair et al. (1986) noted that smoking habits among this cohort did not differ substantially from those of the general population. Checkoway et al. (2015) redefined peak exposures in the referent category to include any exposures <2 ppm of hourly, daily, weekly or monthly frequency as well as exposures > 2 ppm if they occurred hourly or monthly.					peak exposures < 2 ppm as unexposed and to reclassify peak exposures > 2 ppm as unexposed if they were either very rare or very common. **SUMMARY: AML, CML: LOW ↓** (Potential bias ↓)
Beane Freeman et al. (2013); Beane Freeman et al. (2009) United States Cohort study of workers in 10 plants using or producing formaldehyde,	25,619 workers (12% female) followed from plant start-up or first employment. Deaths were identified from the	Individual-level exposure estimates based on job titles, tasks, visits to plants by study industrial hygienists who took 2,000 air samples from representative jobs, and plant monitoring data	Mortality: underlying cause from death certificates, ICD-8. HL: ICD 201 MM: ICD 203 LL: ICD 204 ML: ICD 205. Larynx: ICD 161 NPC: ICD 147	All comparisons adjusted for calendar year, age, sex, and race. Internal analysis adjusted for pay category.	Internal: Poisson regression; RR (95% CI) by exposure categories (4 levels), for peak, average, cumulative exposures.	HL: 27 MM: 59 LL: 37 ML: 48 Larynx: 48 NPC: 11 SNC: 5	 Exposure: Group A Low power for SNC **SUMMARY: SNC: MEDIUM** (Low sensitivity)

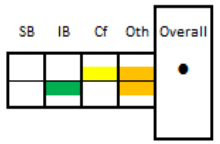
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
follow-up through 2004. Related studies: Initial 10 plant cohort follow-up through 1980 Blair et al. (1987); Blair et al. (1986). Second set of 10 plant follow-ups through 1994 Hauptmann et al. (2004a); Hauptmann et al. (2003). Reanalysis of 1 plant Marsh et al. (2007); Marsh et al. (2002).	National Death Index with remainder assumed to be living. Vital status was obtained for 97.4%. Median follow-up 42 yrs. Average follow-up ≈38.96 yrs. All cancer SMR = 0.93.	from 1960 through 1980. Blinded to outcome. Median cumulative exposure was 0.6 ppm-years (range = 0.0–107.4 ppm-yrs). Co-exposed to antioxidants, **benzene**, carbon black, dyes and pigments, melamine, hexamethylenetetramine, phenols, plasticizers, urea, **wood dust**. No information on smoking; however, according to Blair et al. (1986), "The lack of a consistent elevation for tobacco-related causes of death, however, suggests that the smoking habits among this cohort did not differ substantially from those of the general population."	SNC: ICD 160. Higher survival rates for HL and LL could undercount incident cases, but median follow-up is more than 42 yrs.	For HL, MM, LL, ML: Benzene is a potential confounder but was controlled for. For NPC, SN: Wood dust is a potential confounder but was controlled for. Eleven co-exposures examined as potential confounders, but none were found to be confounders.	Latency was evaluated. External: SMRs (95% CI).		**HL, Larynx, LL, ML, MM, NPC: HIGH**

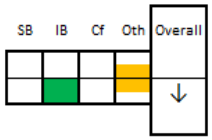
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
		Beane Freeman, 2013, 2452550@@author-year} report that among a sample of 379 cohort members, they "found no differences in prevalence of smoking by level of formaldehyde exposure."					
Bertazzi et al. (1986). Italy Cohort study of Italian chemical workers in plant producing formaldehyde resins.	1,332 male workers ever employed in the plant between 1959 and 1980. Deaths were identified from vital statistics offices. Vital status was 98.6% complete. Average follow-up ≈15.26 yrs.	Individual-level exposure estimates based on occupational histories from the personnel office with supplement information from 350 employed workers alive at the end of follow-up in 1980. 5,731/20,366 (28%) person years were considered to be exposed to formaldehyde.	Death certificates used to determine cause of deaths from nasal cancer (ICD-8).	Controlled for age, sex and calendar time. Styrene is associated with LHP cancers but not URT cancers. Other co-exposures are not known risk factors for this outcome.	SMRs (95% CI). Latency evaluated.	SNC: 0 cases	 Exposure Group B Low power **SUMMARY:** **SNC: LOW ↓** (Low sensitivity Potential bias ↓)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	All cancer SMR = 1.54.	Other exposures included **styrene**, xylene, toluene, and methyl isobutyl ketone.					
Boffetta et al. (1989). United States Nested matched case control of MM within general population cohort. Baseline enrollment in 1982 with bi-annual follow-up in 1984 and 1986.	508,637 men and 676,613 women (57%) in American Cancer Society's Cancer Prevention Study II, with sufficient data on occupation. Loss to follow-up 1.5%. Death certificates for 84% of deceased subjects. Four controls per case were matched for age, sex, ethnic group, and residence.	Self-report from baseline questionnaire occupational history, based on specific question about exposure to formaldehyde (Ever/Never). Other exposures included **asbestos**, chemicals, **acids**, solvents, coal or stone dusts, coal tar, pitch, asphalt, diesel and gasoline exhausts, dyes, pesticides, herbicides, textile fibers/dusts, **wood dust**, **X-rays**, and **radioactive material**.	Mortality: underlying or contributing cause from death certificates MM: ICD-9: 203. Analysis limited to "incident" cases (i.e., had not indicated a history of cancer in baseline questionnaire).	Matching controlled for sex, age, ethnic group, residence, smoking, education, diabetes, X-ray treatment, farming, pesticide, and herbicide exposure. Other co-exposures were not associated with LHP cancers.	Mantel-Haenszel matched OR (95% CI). Latency not evaluated.	MM: 128 (4 exposed)	 Exposure Group C Lack of latency analysis Low power (few exposed cases) **SUMMARY: LOW ↓** (Low sensitivity Potential bias ↓)

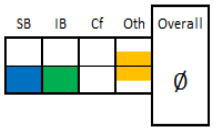
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
Coggon et al. (2014); Coggon et al. (2003) Great Britain Cohort study of British chemical workers in factories using or producing formaldehyde, working before 1940 with follow-up through 2012. Related studies: Initial follow-up through 1981 Acheson et al. (1984). Second follow-up through 1989 Gardner et al. (1993). Third follow-up through 2000: Coggon et al. (2003).	14,008 men in six chemical facilities. Cohort mortality followed from 1941 until December 2012. Vital status was 92% complete. Cause of deaths was known for 99% of 5,185 deaths through 2000. This figure was not provided on 7,378 deaths through 2012. All cancer SMR = 1.10.	Individual level categorical exposure assessment based on employment records evaluated occupational hygienist who classified job titles according to their exposure to formaldehyde based on measurement made after 1970 and workers' recall of irritant symptoms prior to 1970. Background exposure corresponded to <0.1 parts per million (ppm), low exposure to 0.1–0.5 ppm, moderate exposure to 0.6–2.0 ppm, and high exposure to >2.0 ppm. Blinded to outcome. Each worker assigned the highest level of exposure ever experienced (i.e., "ever highly exposed"). Subjects'	Mortality: underlying cause from death certificates, ICD-9. HL: ICD 201 ML: ICD 205 MM: ICD 203. Larynx: ICD 161 MM: ICD 203 NPC: ICD 147 OHPC: ICD 146-149 minus 147 SNC: ICD 160. Note than HL follow-up was through 2000 Coggon et al. (2003). Higher survival rates for HL and LL could undercount incident cases, but follow-up is more than 50 yrs.	Adjusted for calendar year, age. Styrene is associated with LHP cancers but not URT cancers. Asbestos is associated with URT cancers, including laryngeal cancer. Authors stated that the extent of co-exposures was expected to be low. Potential for confounding may be mitigated by low co-exposures.	SMRs (95% CI) by low/moderate and high exposure categories. Latency not evaluated.	NPC: 1 SNC: 2 OHPC: 16 Larynx: 22 HL: 15 MM: 28 ML: 36 Note that HL results is from 2003.	 Exposure: Group B Lack of latency analysis Low power for NPC and SN **SUMMARY:** **NPC, SNC: LOW ↓** (Low sensitivity Potential bias ↓) **HL, Larynx, ML, MM, OHPC: MEDIUM ↓** (Potential bias ↓)

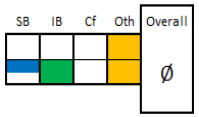
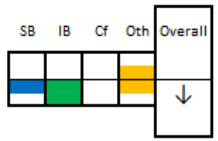
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
		assigned exposure grade may exceed average workplace exposure. Potential low-level exposure to styrene, ethylene oxide, epichlorohydrin, solvents, asbestos, chromium salts, and cadmium.					
Coggon et al. (2014) Great Britain Nested case-control study. Related studies: Initial follow-up through 1981 Acheson et al. (1984). Second follow-up through 1989 Gardner et al. (1993). Third follow-up through 2000 Coggon et al. (2003).	Internal comparison using nested case-control study within cohort with 10 controls per case individually matched by facility, mortality status and age within 2 yrs.	Individual level categorical exposure assessment based on employment records evaluated occupational hygienist who classified job titles according to their exposure to formaldehyde based on measurement made after 1970 and workers' recall of irritant symptoms prior to 1970. Background exposure corresponded to <0.1 ppm, low exposure to 0.1–0.5 ppm, moderate exposure	Incidence or morality: cancer registries and death certificates, ICD-code in effect at time of diagnosis or death. Cases were either incident diagnoses, underlying cause of death, or contributing cause of death. Larynx: 161 MM: ICD 203 NPC: ICD 147 OHPC: ICD 146-149 minus NPC SN: ICD 160.	Matched analysis controlled for facility and age. Styrene is associated with LHP cancers but not URT cancers. Authors stated that the extent of co-exposures was expected to be low. Potential for confounding may be mitigated by low extent of co-exposures.	ORs (95% CI) by low, moderate, high exposure for less than 1 yr, and high exposure for 1 yr or more. Latency evaluated by exposure duration and category at 5 yrs prior to diagnosis or death for each matched set.	Larynx: 53 Pharynx: 28 OHPC: 27 ML: 45 MM: 28	 Exposure Group B Latency evaluation likely to be under-powered to detect any effects beyond a 5-yr period. **SUMMARY: Larynx, ML, MM, OHPC: MEDIUM ↓**

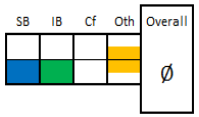
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
		to 0.6–2.0 ppm, and high exposure to >2.0 ppm. Blinded to outcome. Each worker assigned the highest level of exposure ever experienced (i.e., "ever highly exposed"). Subjects' assigned exposure grade may exceed average workplace exposure. Potential co-exposure to **styrene** and solvents.					
Dell and Teta (1995) United States Cohort study of workers in a plastics manufacturing and research and development facility which made phenol-formaldehyde resins, working 1946–1967 with follow-up through 1988.	5,932 white men employed for at least 7 mos. Vital status was 94% complete. Death certificates obtained for 98%.	Individual exposure measures not evaluated. Only 111 men (2%) had work assignments involving formaldehyde. However, as the plant manufactured and used formaldehyde since 1931, a larger percentage may have	Mortality: underlying cause from death certificates, ICD version in effect at time of death. MM: ICD 203.	Adjusted for sex, race, age, and calendar-year. Asbestos is not associated with LHP cancers. Benzene and styrene were not evaluated as potential confounders and	SMRs (95% CI) by major department. Latency evaluated with exposure lag times of 10 and 15 yrs.	MM: 8 NPC: 0	 Exposure: Group C Confounding possible Low power due to rarity of exposure **SUMMARY for MM: LOW**

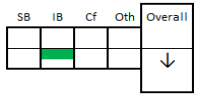
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	Average follow-up 32 yrs. All cancer SMR = 1.02.	actually been exposed. Variation in presumed exposure by department and pay status. Co-exposures: acrylonitrile, **asbestos**, **benzene**, carbon black, epichlorohydrin, PVC (vinyl chloride), **styrene,** and toluene.		would likely be positively correlated with formaldehyde exposure. Potential for confounding is unknown but could have inflated the observed effect.			(Potential biases) **SUMMARY for NPC: Not informative** (Low sensitivity Potential biases)
Edling et al. (1987b) Sweden Cohort study of workers in a production plant making abrasives bound with formaldehyde resins, working 1955 to 1981 with follow-up through 1983.	521 male workers employed at least 5 yrs. Vital status was 97% complete. All cancer SMR = 0.93.	Whole cohort assumed to be exposed with some individual's exposed to high peak exposures. Manufacture of grinding wheels bound by formaldehyde resins exposed company workers to 0.1–1 mg/m^3 formaldehyde. 59 workers (11%) had intermittent	Incidence (ICD-8), from National Cancer Registry. MM: ICD-203.	Controlled for sex, age, and calendar-year-specific mortality rates. Co-exposures are not known risk factors for this outcomes.	SIRs (95% CI). Latency not evaluated.	MM: 2	 Exposure: Group B Latency not evaluated Low power **SUMMARY:** **MM: LOW ↓** (Low sensitivity potential bias ↓)

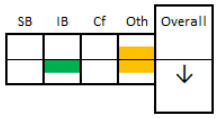
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
		heavy exposures to formaldehyde with peaks up to 20–30 mg/m^3. Co-exposed to aluminum oxide and silicon carbide.					
Fryzek et al. (2005) United States Cohort mortality study of workers in motion picture film processing, working 1960 to 2000, with follow-up through 2000.	2,646 workers (11% female) employed at least 3 mos. 178 workers (7%) excluded for missing work histories or work outside the study period. Vital status obtained for 99.7%; cause of death data for 655 of 666 decedents (98.3%). Average length of follow-up ≈20.58 yrs.	Individual-level occupational histories were used to classify workers in job families matched to past industrial hygiene surveys conducted in house and by state program. Formaldehyde used in "film developing" and possibly in 'maintenance'. Personal and area sample averaged 0.28–0.29 ppm with range 0.06–0.52. Co-exposures included methanol, methyl chloroform, **perchloroethylene**, and hydroquinone.	Mortality: underlying cause from death certificates. HL: ICD-9 201 MM: ICD-9 203. Higher survival rates for HL could undercount incident cases, but average follow-up is more than 20 yrs.	Controlled for age, sex, race, and time period. Perchloroethylene may be a risk factor for multiple myeloma as may hydroquinone which is a metabolite of benzene, a known cause of LHP cancers. Potential for confounding is unknown but could have substantially inflated the observed effect due to the high correlation of these exposures	SMRs (95% CI). Decade of exposure, duration of exposure and time since first exposure were evaluated. Latency was evaluated as time since first exposure.	HL: 0 MM: 2	 Exposure: Group B Confounding likely Low power **SUMMARY: NOT INFORMATIVE** Critical limitation: Confounding

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	All cancer SMR = 1.1.			with formaldehyde.			
Hall et al. (1991) Great Britain Cohort study of British pathologists. Related studies: Initial follow-up through 1973 Harrington and Shannon (1975) Second follow-up through 1980 Harrington and Oakes (1984).	4,512 pathologists from the Royal College of Pathologists and the Pathological Society of Great Britain from 1974–1987. Deaths among those >85 yrs were censored. Vital status was obtained from the census, a national health registry, and other sources (100%). Cause of death data for 222 of 231 individuals (96.5%).	As a profession, pathologists were highly exposed to formaldehyde as a main ingredient in tissue fixative. NIOSH (*Industry Selection for Determination of Extent of Exposure,* 1979) has reported mean formaldehyde concentrations of 4.35 ppm with range (2.2–7.9). Co-exposures may have included: phenol, methyl alcohol, glutaraldehyde, mercury, arsenic, zinc, and **ionizing radiation**.	Mortality: cause of death = Hodgkin lymphoma, ICD 8: code 201. Higher survival rates for HL could undercount incident cases, but maximum follow-up is 13 yrs with 5% mortality during follow-up.	Controlled for age, sex, and calendar year. Chemical co-exposures are not known risk factors for this outcome. Radiation exposure likely to be poorly correlated with formaldehyde.	SMRs (95% CI) developed from the English and Welsh populations. Latency not evaluated.	HL: 1 Low power due to the rarity of cases.	 Selection: Extremely healthy population with overall cancer SMR of 0.44 Exposure: Group B Lack of latency analysis Low power **SUMMARY: NOT INFORMATIVE** Critical limitation: Selection bias

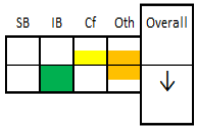
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	All cancer SMR = 0.44.						
Hansen et al. (1994) Denmark Cohort study of workers at a Danish pharmaceutical plant.	10,889 employees (51% women) ever employed 1964–1988 at a pharmaceutical plant. Cases were extracted from the Danish Cancer Registry. All cancer SIR (men)=0.95 All cancer SIR (women) = 1.16.	No individual-level exposures estimated: whole cohort assumed to be exposed. Formaldehyde was one of many exposures in this industry but not a main ingredient or product. Co-exposures may have included **asbestos**, antibiotics, chloroform, dichloromethane, enzymes, **ethylene oxide**, glucagon heparin, insulin, silica, sex hormones, sodium saccharin, and synthetic agents.	Incidence: cases from Danish Cancer Registry classified according to ICD-7. HL: ICD 201 MM: ICD 203. Higher survival rates for HL could undercount incident cases, although average follow-up is 13 years.	Controlled for age, sex, and calendar year. Asbestos is associated with URT cancers. Ethylene oxide is associated with LHP cancers. Neither were evaluated as potential confounders. Potential for confounding is mitigated by low formaldehyde exposure and likely low correlation with asbestos and ethylene oxide.	SIRs (95% CI). Latency not evaluated.	HL: 4 Larynx: 5 MM: 0 Low power due to the rarity of cases and low confidence in formaldehyde exposure.	 Potential selection: Mortality for HL Exposure Group D Latency not evaluated Low power **SUMMARY: NOT INFORMATIVE** Critical limitation: Information bias (minimal exposure)
Hansen and Olsen (1995). Denmark Cohort study of Danish men, URT cancers diagnosed 1970–1984.	2,041 men with incident cancer whose longest work experience occurred at least 10 yrs	Individual occupational histories including industry and job title established through company tax records.	Incident cases identified in Danish Cancer Registry (ICD-7). NPC: 146 SNC: 160 Larynx: 161	Controlled for age, sex, and calendar time. Sinonasal cancer risk was evaluated	SPIRs (95% CI) (Standardized proportionate incidence ratio) - proportion of cases for a given cancer in formaldehyde-	NPC: 4 SNC: 13 Larynx: 32 HL: 12	 Potential selection: mortality for HL

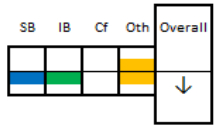
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	before cancer diagnosis. Cases matched with employment records from pension fund (72%) with remainder being self-employed, pensioners, and unemployed. External comparison with general population. Average follow-up ≈13 yrs.	Considered exposed if worked in plant with more than 1 kg formaldehyde used per employee per year. Very crude exposure assessment. No information on co-exposures except for **wood dust**.	HL: 201. Higher survival rates for HL could undercount incident cases, although average follow-up is approximately 13 yrs.	controlling for wood dust. While other co-exposures were not evaluated, the overall correlation between co-exposures in multiple occupational industries is likely to be low.	associated companies relative to the proportion of cases for the same cancer among all employees in Denmark. Latency addressed by inclusion criteria.		Exposure Group D Low power for NPC **SUMMARY:** **HL, Larynx, NPC, SNC: LOW ↓** (Potential bias ↓)
Harrington and Oakes (1984). Great Britain Second cohort study of British pathologists. Related studies: Initial follow-up through 1973	2,720 pathologists from the Royal College of Pathologists and the Pathological Society of Great Britain	As a profession, pathologists were highly exposed to formaldehyde as a main ingredient in tissue fixative. NIOSH (*Industry Selection for Determination of Extent of Exposure,*	Mortality: cause of death sinonasal cancer.	Controlled for age, sex, and calendar year. Radiation exposure likely to be poorly correlated with formaldehyde.	SMRs (95% CI) developed from the English and Welsh populations. Latency not evaluated.	SNC: 0 Low power due to the rarity of cases.	 Selection: Extremely healthy population with overall cancer SMR of 0.61 Exposure: Group B Lack of latency analysis

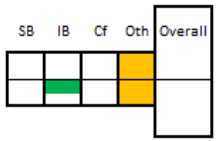
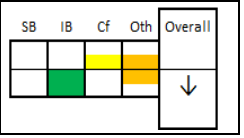
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
Harrington and Shannon (1975) Third follow-up through 1987 Hall et al. (1991).	from 1974–1980. Deaths among those >85 yrs were censored. Vital status was obtained from the census, a national health registry, and other sources (100%). 96% of death certificates were obtained with 91 reporting a cause of death. All cancer SMR = 0.61.	1979) has reported mean formaldehyde concentrations of 4.35 ppm with range (2.2–7.9). Co-exposures may have included: phenol, methyl alcohol, glutaraldehyde, mercury, arsenic, zinc, and **ionizing radiation**.		Chemical co-exposures are not known risk factors for this outcome.			Low power **SUMMARY: NOT INFORMATIVE** Critical limitation: Selection bias
Hauptmann et al. (2009). United States Nested case-control study within extension of embalmers cohorts	Embalmers (8% women) from national and state funeral directors associations and licensing	Individual level, based on lifetime work practices and exposures to formaldehyde obtained by interview with next of kin or co-workers	Mortality: underlying cause from death certificates, ICD-8. MM: ICD 203 LL: ICD 204 ML: ICD 205.	Controlled for date of birth, age at death, sex, data source, and smoking. Radiation exposure likely	Logistic regression, OR (95% CI) by exposure categories (4 levels) for duration, number of	ML: 34 (17 acute) MM: *n* cases not reported but must be greater than 5 due to size of se(ln(OR)).	 Exposure: Group A Latency not evaluated for LL or MM

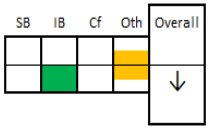
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
described in Hayes et al. (1990); Walrath and Fraumeni (1984, 1983).	boards. Died 1960–1986. Participation rate of case interviews was 220/228 (96%) and 265/282 eligible controls (94%). Controls randomly selected from individuals in the funeral industry whose deaths were attributed to other causes. Controls stratified to be similar to data source, sex, and dates of birth and death (5-yr intervals).	(96% of cases and controls) with information on occupational exposure resulting from embalming. Interviewers blinded to outcome. Exposure levels assigned based on laboratory reconstruction of exposures for specific work practices. Co-exposures may have included: phenol, methyl alcohol, glutaraldehyde, mercury, arsenic, zinc, and **ionizing radiation**.	Higher survival rates for HL could undercount incident cases, but average follow-up is more than 39 yrs (485 cases and controls/19,104 person-yrs).	to be poorly correlated with formaldehyde. Chemical co-exposures are not known risk factors for this outcome.	embalmings, cumulative exposure, average intensity, time-weighted average, and peak exposure measures. Analyses of duration of exposure for MM is proxy for latency.	LL: 99 NPC: 4	**SUMMARY:** **ML: HIGH** **LL, MM: MEDIUM** ↓ (Potential bias ↓)
Hayes et al. (1990) United States	4,046 deceased male embalmers	Individual exposure measures not derived. Occupation	Mortality: underlying cause of death from death certificates, ICD-8;	Controlled for calendar year, age, sex, and race.	PMR (95% CI). Latency not evaluated.	HL: 3 Larynx: 7 LL: 7 ML: 24	

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
Cohort study of embalmers. Related study: Hauptmann et al. (2009)	and funeral directors, derived from state licensing boards and funeral director who died during 1975–1985 and a death certificate could be obtained. Death certificates obtained for 79% of potential study subjects. The 21% missing death certificates considered to missing at random because all embalmers were considered to be exposed to formaldehyde.	confirmed from death certificates. Separate study estimated personal formaldehyde exposures from 0.98 ppm (high ventilation) to 3.99 ppm (low ventilation), with peaks up to 20 ppm. Co-exposures may have included: phenol, methyl alcohol, glutaraldehyde, mercury, arsenic, zinc, and **ionizing radiation**.	ICD 201 = HL ICD 203 = MM ICD 204 = LL ICD 205 = ML. Higher survival rates for HL and LL could undercount incident cases, and median follow-up is unknown.	Radiation exposure likely to be poorly correlated with formaldehyde. Chemical co-exposures are not known risk factors for this outcome.		MM: 20 NPC: 4 SNC: 0 Possible undercounting of cases due to abbreviated death certificate search.	Exposure: Group A Latency not evaluated Low power for HL, NPC, SNC **SUMMARY:** **Larynx, LL, ML, MM: MEDIUM ↓** (Potential bias ↓) **HL, NPC, SNC: LOW ↓** (Potential bias ↓ low sensitivity)

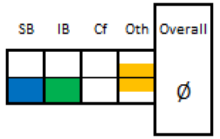
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	All cancer PMR (white) = 1.07 (nonwhite) = 1.08.						
Jakobsson et al. (1997) Sweden Cohort study of workers grinding stainless steel.	727 male employees of 2 plants producing stainless steel sinks and sauce pans employed at least 1 yr during 1927–1981 with minimum 15-yr follow-up. Of 823 original workers, 23 (3%) could not be identified, 12 died or emigrated before 1952 (1%), and 61 did not exceed the 15 yr waiting	No individual exposure measures. Presumed exposure was to phenol-formaldehyde resins on ribbons or plates in grinding workers. Co-exposures may have included **chromium**, **nickel**, and abrasive dusts including silicon carbide, aluminum oxide, silicon dioxide, and clay. No wood dust exposures.	Incidence: cases from Swedish Tumor Registry SN ICD-7 160.	Adjusted for sex, age, and calendar year. Nickel and chromium are associated with URT cancers and would likely be positively correlated with formaldehyde exposure. Potential for confounding is unknown but could have inflated the observed effect. Other co-exposures are not known risk factors for these outcomes.	SIRs (95% CIs). Latency addressed by enforcing a 15-yr waiting period to begin observation.	Larynx:1 SNC: 0 Low power due to the rarity of cases.	 Exposure Group D Confounding possible for laryngeal cancer Low power **SUMMARY: Larynx, SNC: LOW ↓** (Potential bias ↓ low sensitivity)

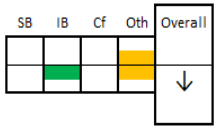
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	period. No further losses to follow-up. All cancer SIR = 0.9.						
Levine et al. (1984b) Canada Cohort study of undertakers.	1,477 male undertakers first licensed during 1928–1977 with mortality follow-up from 1950–1977. Vital status was 96% complete with cause of death available for 94%. Average follow-up 25 yrs. All cancer SMR = 0.87.	As a profession, undertakers/embalmers were highly exposed to formaldehyde as a main ingredient in tissue fixative. Kerfoot and Mooney (1975) reported mean formaldehyde concentrations for embalmers in funeral homes of 0.74 ppm with range (0.09–5.26). Co-exposures may have included: phenol, methyl alcohol, glutaraldehyde, mercury, arsenic, zinc, and **ionizing radiation**.	Mortality: underlying cause from death certificates (ICD-8). Nose, middle ear, sinuses: 160 Larynx: 161.	Controlled for calendar year, age, and sex. Radiation exposure likely to be poorly correlated with formaldehyde. Chemical co-exposures are not known risk factors for this outcome.	SMR, 95% CI. Latency was not evaluated for these endpoints.	SNC: 0 Larynx: 1 Low power due to the rarity of cases.	 Potential selection: Healthy worker effect possible Exposure Group A Latency was not evaluated Low power **SUMMARY: Larynx, SNC: LOW ↓** (Potential bias ↓ low sensitivity)

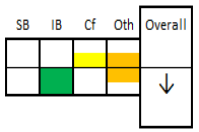
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
Li et al. (2006) China Nested case-cohort study within a cohort study of textile workers.	67 women diagnosed during 1989–1998 with nasopharyngeal cancers were identified in a cohort of 267,400 female textile workers born during 1925–1958. Nine additional cases (12% of total) were excluded due to lack of occupational histories. 3,188 controls randomly selected from the cohort frequency matched by age.	Individual level, based on job exposure matrix developed for this industry/setting (unclear extent of industrial hygiene specifically for formaldehyde). No historical measurements of exposures. No cases were classified as exposed and only 10/3,188 controls (0.3%) were classified as exposed. EPA considered the potential for formaldehyde exposure to be exceedingly low. Co-exposed to cotton **dust**.	Incidence or mortality. Diagnosis of nasopharyngeal cancer or sinonasal cancer reported to a cancer and death registry operated by the Shanghai Textile Industry Bureau. NPC: ICD-9 147 SN: ICD-9 160.	Controlled for age and sex. Dusts could be a potential confounder but due to the rarity of formaldehyde exposure the correlation would be minimal.	Cox proportional hazards modeling adapted for case cohort design. Hazard ratios (95% CI). Duration and latency were not evaluated.	NPC: 10 No cases exposed. Very low power due to the rarity of exposure.	 Exposure Group B Very low power due to the rarity of exposure **SUMMARY: NOT INFORMATIVE** (Very low sensitivity potential bias ↓)
Malker et al. (1990) Sweden	471 employed men with	No individual exposure measures.	Incident cases identified in Swedish Cancer-	Controlled for age and region.	SIRs (95% CI).	NPC: 12	

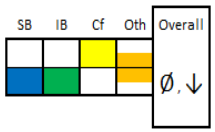
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
Cancer registry-based study, NPC diagnosed 1961–1979.	incident NPC cancer.	Occupations and industries with potential exposure to formaldehyde: bookbinders, fiberboard makers, textile workers, furniture makers, chemical workers, physicians, foundry workers, biologists, tanners, and skin processors, worker employed in veneer and plywood plants and in sugar processing plants. Co-exposure information not provided.	Environment Registry. Microscopic confirmation obtained for 99.6% of NPC cases. 48% squamous cell carcinomas, 37% unspecified carcinomas, 5% transitional cell carcinomas, and 3% adenocarcinomas.	Variation in exposure was not evaluated. Co-exposures were also not evaluated. Fiberboard workers are also exposed to wood dust. Wood dust is associated with URT cancers and would likely be positively correlated with formaldehyde exposure. Potential for confounding is unknown but could have inflated the observed effect.	Latency not evaluated.		Exposure Group D Latency not evaluated Confounding possible Low power for any one occupation which may be potentially exposed **SUMMARY:** **NPC: Low ↓** (Potential bias ↓ low sensitivity)
Marsh et al. (2007); Marsh et al. (2002) United States Nested case-control study within a cohort	7,328 workers employed at a formaldehyde using plant in Connecticut followed from	Worker-specific exposure measures from job exposure matrix based on available sporadic plant monitoring	Mortality: oropharyngeal code ICD-9: 146. Hypopharyngeal code ICD-9: 148.	Controlled for age, race, sex, and time period. Comparison was with U.S. death	SMR (95%CI) Secondary analysis for NPC. EPA derived SMRs for the	Oro: 5 Hypo: 3 Low power due to the rarity of cases.	 Exposure Group B

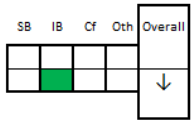
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
study of workers in one plant using formaldehyde. Related studies: Initial 10 plant cohort follow-up through 1980 Blair et al. (1987); Blair et al. (1986). Second set of 10 plant follow-ups through 1994 Hauptmann et al. (2004a); Hauptmann et al. (2003). Third set of 10 plant follow-ups through 2004 Beane Freeman et al. (2013); Beane Freeman et al. (2009).	1945 through 1998. Vital status was identified from the National Death Index, private businesses, or state and local agencies, and was 98.4% complete; cause of death data for 95% of 2,872 deaths. Average follow-up ≈32.89 yrs. All cancer SMR = 1.08.	data from 1965–1987, job descriptions, and verbal job descriptions by plant personnel and industrial hygienists. Exposure assessment did not include the same industrial hygiene sampling conducted by Stewart et al. (1986) used in the Beane Freeman et al. (2013); Beane Freeman et al. (2009) analyses which included this plant. Exposure estimates were on average 10 times lower than those of other studies in this plant (Beane Freeman et al., 2013; Beane Freeman et al., 2009; Blair et al., 1986). From Beane Freeman et al.	Nasopharyngeal code ICD-9: 147. Pharyngeal ICD-9: 146–149. Death certificates used to determine underlying cause of death according to the ICD codes at time of death. Histological typing not reported.	rates and with death rates in 2 counties. Benzene is not associated with URT cancers. Potential confounders were evaluated but only smoking was found to be a potential confounder and was controlled for. Co-exposures to pigments and particles were evaluated and were found not to be confounding. Marsh et al. (2002) attempted to evaluate smoking but data were incomplete. No other potential confounders were evaluated.	combination of oropharyngeal, hypopharyngeal and unspecified pharyngeal cancer by NPC cases from all pharyngeal cancers. Latency not evaluated.	NPC: cases included in Beane Freeman et al. (2013).	Latency not evaluated Low power **SUMMARY:** Oro- alone & Hypo- alone: **LOW** (Potential bias ↓ low sensitivity) OHPC together: **MEDIUM (**Potential bias ↓**)**

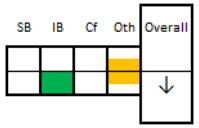
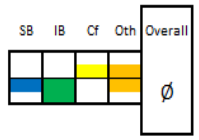
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
		(2013); Beane Freeman et al. (2009): Co-exposed to antioxidants, **benzene**, carbon black, dyes and pigments, melamine, hexamethylenetetramine, phenols, plasticizers, urea, **wood dust**.		Beane Freeman et al. (2013); Beane Freeman et al. (2009)evaluated 11 potential confounders among a set of 10 plants that included this one and did not find any confounding.			
Matanoski (1989) United States Prospective mortality cohort study with two external comparison groups.	3,644 deceased male pathologists, derived from membership rolls of multiple professional societies. Mortality followed through 1978. Death certificates obtained for 94% of potential study subjects, 3%	As a profession, pathologists were highly exposed to formaldehyde as a main ingredient in tissue fixative. NIOSH (*Industry Selection for Determination of Extent of Exposure,* 1979) has reported mean formaldehyde concentrations of 4.35 ppm with range (2.2–7.9). Co-exposures may have included: phenol, methyl alcohol,	Mortality: death certificates and obituary notices used to determine cause of death from Hodgkin lymphoma (ICD-8: 201). Higher survival rates for HL could undercount incident cases, although median follow-up is probably more than 15 yrs since follow-up was from the early 20th century through 1978.	Controlled for sex, race, age, and calendar-year-expected deaths from the U.S. population and psychiatrists. Variation in exposure was not evaluated. Radiation exposure likely to be poorly correlated with formaldehyde. Chemical co-exposures are not known risk	SMRs (95% CI). Latency not evaluated.	HL: 2 cases total Low power due to the rarity of cases.	 Selection: Healthy worker effect probable with overall cancer SMR of 0.78. Exposure: Group B Latency not evaluated Low power **SUMMARY: NOT INFORMATIVE** Selection and information biases

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	from obituary notices and 3% presumed dead. All cancer SMR = 0.78.	glutaraldehyde, mercury, arsenic, zinc, and **ionizing radiation**.		factors for this outcome.			
Meyers et al. (2013) United States Prospective cohort mortality study. Related studies: Initial cohort follow-up Stayner et al. (1988), Second follow-up Pinkerton et al. (2004)	Workers in 3 U.S. garment plants (*n*=11,043) in Georgia and Pennsylvania exposed for at least 3 mos (82% female). Vital status was followed through 2008 with 99% completion. Causes of death were obtained for 3,904 (99.7%) of the 3,915 identified deaths. Average follow-up ≈37.52 yrs.	Individual-level exposure estimates for 549 randomly selected workers during 1981 and 1984 with 12–73 within each department. Formaldehyde levels across all departments and facilities were similar. Exposures ranged from 0.09–0.20 ppm. Overall geometric mean concentration of formaldehyde was 0.15 ppm, (GSD 1.90 ppm). Area measures showed constant levels without peaks. No other chemical exposures were	Mortality: death certificates used to determine the underlying cause of death (ICD-10): NPC: C11 OHPC: C09-C10, C12-C14 SN: C30-31 Larynx: C32. HL:C81 LL: C91.0-91.3, C91.5-91.9 ML: C92 MM: C88.7, 88.9, 90. Higher survival rates for HL could undercount incident cases, but average follow-up is more than 37 yrs Histological typing not reported.	Adjusted for sex, age, race, and calendar-year specific US mortality rates. No other chemical exposures were identified by the industrial hygiene surveys that could influence the findings.	SMRs (95% CI), by exposure categories (3 levels) for duration, time since first exposure measures. SRRs (95% CI) (internal comparison), by 3 categories of duration of exposure. Latency effects were examined for leukemia.	NPC: 0 OHPC: 6 SNC: 0 Larynx: 4 ML; 21 (14 acute; 5 chronic) LL: 6 HL: 4 MM: 23	 Exposure Group A Latency for leukemia only Low power for NPC, SNC, Larynx, HL **SUMMARY: Larynx, NPC, SN: LOW ●** (Potential bias ↓ low sensitivity) **HL, MM, OHPC: MEDIUM↓** (Potential bias ↓) **LL, ML: HIGH**

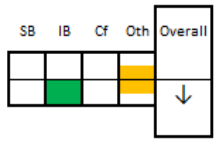
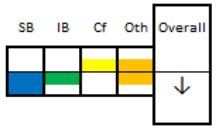
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	All cancer SMR = 0.96.	identified by the industrial hygiene surveys. There was no information on smoking in this analysis, however, according to Stayner et al. (1988), "the overall prevalence of cigarette smokers was 29.4%. In plant 1 the prevalence was 26.6%, in plant 2 it was 33.5%, and in plant 3 it was 29.4%. These figures are similar to those reported in a 1980 survey of adult Americans, in which 29.2% of females and 38.3% of males over the age of 20 were current cigarette smokers [NCHS, 1985]."					
Ott et al. (1989) United States (West Virginia)	29,139 male workers followed from 1940–1978. Loss to	Individual-level exposure classification based on company records of work assignments	Mortality: underlying cause from death certificates, ICD	Unconditional logistic regression. Controlled for sex and age.	OR (95% CI). Analyses conducted with a 5-yr exposure	MM: 20 ML: 39 LL: 18	 Exposure Group B

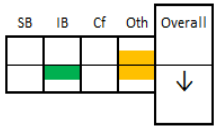
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
Nested case-control study within two chemical manufacturing plants.	follow-up 3.6%. 95.4% of death certificates obtained. Frequency matching of controls (5:1) from the total employee cohort according to a group-matched incidence density sampling design.	linked to records on department usage of formaldehyde. Exposures during 1940 to 1978. 21 different chemicals were evaluated including **benzene** with much cross exposure.	version in effect at time of death. Higher survival rates for LL could undercount incident cases, but average follow-up is likely more than 15 yrs as follow up was initiated in 1940 and ceased in 1978.	Controlling for age did not change results. Benzene was not evaluated as a potential confounder and may be positively correlated with formaldehyde exposure. Potential for confounding is unknown but could have inflated the observed effect. Potential for confounding may be mitigated by rarity of co-exposures among cases.	lag. Limited adjustment for latency.	≤2 exposed cases for each endpoint Low power due to the rarity of exposure.	Latency evaluation likely to be under-powered to detect any effects beyond a 5-yr period. Confounding possible Low power due to rarity of exposure **SUMMARY:** **LL, ML, MM: LOW ↓** (Low sensitivity potential bias ↓)
Robinson et al. (1987) United States	Plywood mill workers (*n*=2,283) employed at least 1 yr	Individual exposure measures not derived.	Mortality: underlying cause from death certificates (ICD-7) HL: 201	Adjusted for sex, age, race, and calendar-year-specific U.S. mortality rates.	SMRs (90% CI). Latency not evaluated.	MM: 3 cases HL: 2 cases (2 cases, whole cohort of mill workers; 2	 Selection: Healthy

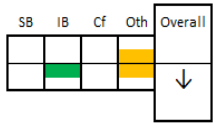
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
Prospective cohort mortality study.	during 1945–1955 followed for mortality until 1977 with vital status for 98% and death certificates for 97% of deceased. Average follow-up ≈25.22 yrs. All cancer SMR = 0.7.	Presumed exposure to formaldehyde-based glues used to manufacture and patch plywood. Co-exposure to carbon disulfide, **pentachlorophenol**, wood dust.	MM: 203. Higher survival rates for HL could undercount incident cases, but average follow-up is more than 25 yrs.	Some exposed workers also exposed to pentachlorophenol for more than 1 yr. EPA concluded that pentachlorophenol is likely to be carcinogenic based on strong evidence from epidemiologic studies of increased risk of MM. Potential for confounding is unknown but could have inflated the observed effect for MM but not for HL.		cases, subcohort of exposed workers)	worker effect probable with overall cancer SMR of 0.7. Exposure Group D Latency not evaluated MM likely confounded by pentachlorophenol Low power **SUMMARY:** **MM: Not informative,** **(Low sensitivity, likely confounding)** **HL: LOW ↓** (Low sensitivity potential bias ↓)
Saberi Hosnijeh et al. (2013) Europe Prospective cohort study.	241,465 men and women recruited from 10 European countries during 1992–	Occupational histories obtained by questionnaire about ever working in any of 52 occupations considered to be at high risk of	Incident primary leukemias identified from cancer registries, health insurance records, pathology registries and	Controlled for age, sex, smoking, alcohol, physical activity, education, BMI, family history of cancer, country,	Proportional hazards regression; HRs (95% CI). Latency was not evaluated.	LL: 67/225 exposed ML: 49/179 exposed	 Exposure Group C Latency was not evaluated

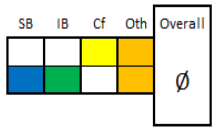
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	2000. Participants were predominantly ages 35–70 at recruitment and were followed up through 2010.	developing cancer. Occupational exposures estimated as "high," "low," and no exposure by linking to a JEM.	contact with subjects of their next of kin.	other occupational exposures, and radiation.			**SUMMARY:** **LL, ML: LOW ↓** (Potential bias ↓)
Siew et al. (2012) Finland National cohort study.	All Finnish men born during 1906–1945 who participated in census and were employed in 1970 (*n*=1.2 million). Cancer cases identified by national registry during 1971–1995.	Occupational history from census records were linked to the national JEM to code each cohort member with "any" exposure to formaldehyde or "none." Only some use of "industry" information. 3% of NPC cases exposed 5% of SNC cases exposed Co-exposure wood dust was collected.	Diagnosis of cancer reported to the Finnish Cancer Registry.	Controlled for age, sex, socioeconomic status, smoking, and wood dust.	SIRs (95% CI). A 20-yr latency period was assumed.	NPC: 149 SNC: 167. Baseline incidence of NPC in this population is the lowest in the world.	 Exposure Group D Low power due to rarity of exposure **SUMMARY:** **NPC, SNC: LOW ↓** (Potential bias ↓)
Solet et al. (1989) United States Proportionate mortality study of	201 white male pulp and paper producing workers who	Occupational history from union records identified workers in the pulp and paper producing jobs.	Mortality: underlying cause from death certificate submitted to the	Controlled for age, sex, race, age at death, and calendar time.	PMRs (95% CI). Latency not evaluated.	HL: 1 case Low power due to the rarity of cases.	

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
pulp and paper workers.	died during 1970–1984 and had at least 10 yrs of experience in the industry. All cancer PMR = 1.31.	Formaldehyde is known to be an exposure for pulp and paper mill workers: job-specific exposures range from 0.2 to 1.1 ppm with peaks as high as 50 ppm (Korhonen et al., 2004). From Band et al. (1997), co-exposed to arsenic, **chlorophenols**, **sulfuric acid mists**, and chloroform. According to a review Kauppinen et al. (1997) co-exposures to **dioxin** or **perchloroethylene** are also possible.	Union Pension Fund. HL: ICD-8 201. Higher survival rates for HL could undercount incident cases, but average follow-up is probably more than 15 yrs because workers had to have at least 10 yrs of experience in the industry.	Confounding not evaluated. Potential confounders for these outcomes include chlorophenols, acids mists, dioxin, and perchloroethylene, which are likely to have been positively correlated with formaldehyde exposure. Other co-exposures are not known risk factors for these outcomes. Potential for confounding is unknown but could have inflated the observed effect.			Potential selection: mortality for HL Exposure Group D Latency not evaluated Confounding possible Low power **SUMMARY: NOT INFORMATIVE** Critical limitation: (multiple potential biases and uncertainties)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
Stellman et al. (1998) United States General population cohort. Baseline enrollment in 1982; follow-up through 1988.	317,424 men enrolled in the American Cancer Society's Cancer Prevention Study II in 1982. Follow-up was 98% complete. Median follow-up 6 yrs. Average follow-up ≈5.79 yrs.	Individual level, based on questionnaire response (Yes/No) on formaldehyde exposure. Excludes wood-related occupations. Specific co-exposures included **asbestos** and **wood dust**.	Mortality: death certificates, MM: ICD-9 203.	Controlled for age, sex, and smoking. Co-exposures are not associated with LHP cancers.	Poisson regression (internal comparison) RRs (95% CI). Latency not evaluated.	MM: 14 (4 exposed) Low power dues to the rarity of exposure.	 Exposure Group C Latency not evaluated Low power **SUMMARY: LOW ↓** (Low sensitivity potential bias ↓)
Stroup et al. (1986) United States Retrospective cohort mortality study.	2,239 deceased white male anatomists identified from professional societies who died during 1925–1979. 91% of death certificates of	As a profession, anatomists were highly exposed to formaldehyde as a main ingredient in tissue fixative. Akbar-Khanzadeh and Mlynek (1997) reported mean formaldehyde concentrations in anatomy	Mortality: underlying cause from death certificates (ICD-8), HL: 201 Larynx: 161 ML: 205 SNC: 160. Higher survival rates for HL could undercount incident cases, but	Controlled for calendar year, age, sex, race compared with U.S. population. Radiation exposure likely to be poorly correlated with formaldehyde.	SMR (95% CI). Latency not evaluated.	HL: 0 Larynx: 1 ML: 5 (1 acute, 3 chronic, 1 unspecified) SNC: 0 Low power due to the rarity of cases.	 Selection: Healthy worker effect probable with overall cancer SMR of 0.64. Exposure Group A Latency not evaluated

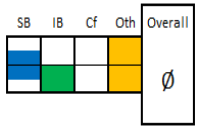
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	known deceased obtained. Average follow-up ≈22.52 yrs. All cancer SMR = 0.64.	laboratories of 1.9 ppm with range (0.3–4.5). Co-exposures may have included: phenol, methyl alcohol, glutaraldehyde, mercury, arsenic, zinc, and **ionizing radiation**. Anatomists may also be co-exposed to stains, **benzene**, toluene, xylene, chlorinated hydrocarbons, dioxane, and osmium tetroxide.	average follow-up is more than 22 yrs.	Benzene not evaluated as potential confounder but is a risk factor for ML. Potential for confounding is unknown but could have inflated the observed effect.			Confounding possible for ML Low power **SUMMARY: HL, Larynx, ML, SNC: LOW ↓** (Low sensitivity potential bias ↓)
Walrath and Fraumeni (1983) United States Cohort mortality study. Related study: Hauptmann et al. (2009)	1,132 deceased white male embalmers identified from NY state license board. Died 1925–1980. Death certificates	As a profession, embalmers were highly exposed to formaldehyde as a main ingredient in tissue fixative. Kerfoot and Mooney (1975) reported mean formaldehyde concentrations for embalmers in funeral homes of	Mortality: underlying cause from death certificates (ICD-8) HL: 201 LL: 204 ML: 205. Higher survival rates for HL and LL could undercount incident cases, but average follow-up	Controlled for calendar year, age, sex, and race. Radiation exposure likely to be poorly correlated with formaldehyde. Chemical co-exposures are	PMR, 95% CI. Latency was not evaluated for these endpoints.	HL: 7 Larynx: 2 LL: 4 ML: 7 SNC: 0 Low power for LL due to the rarity of cases.	 Exposure Group A Latency was not evaluated. Low power for larynx, LL, SNC **SUMMARY:**

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	obtained for 75%. The 25% missing death certificates considered to missing at random because all embalmers were considered to be exposed to formaldehyde. All cancer PMR = 1.11.	0.74 ppm with range (0.09–5.26). Co-exposures may have included: phenol, methyl alcohol, glutaraldehyde, mercury, arsenic, zinc, and **ionizing radiation**.	is likely more than 15 yrs as follow up was initiated in 1925 and ceased in 1980.	not known risk factors for this outcome.			**Larynx, LL, SNC: LOW ↓** (Low sensitivity potential bias ↓) **HL, ML: MEDIUM ↓** (Potential bias ↓)
Walrath and Fraumeni (1984) United States Cohort mortality study. Related study: Hauptmann et al. (2009)	1,007 deceased white male embalmers identified from CA state license board. Died 1925–1980. Death certificates obtained for 100%.	As a profession, embalmers were highly exposed to formaldehyde as a main ingredient in tissue fixative. Kerfoot and Mooney (1975) reported mean formaldehyde concentrations for embalmers in funeral homes of 0.74 ppm with range (0.09–5.26).	Mortality: underlying cause from death certificates (ICD-8) HL: 201 LL: 204 ML: 205. Higher survival rates for HL and LL could undercount incident cases, but average follow-up is likely more than 15 yrs as follow up was initiated in	Controlled for calendar year, age, sex, and race. Radiation exposure likely to be poorly correlated with formaldehyde. Chemical co-exposures are not known risk factors for this outcome.	PMR, 95% CI. Latency was not evaluated for these endpoints.	ML: 8 Larynx: 2 LL: 4 HL: 0 SNC: 0 Low power due to the rarity of cases.	 Exposure Group A Latency was not evaluated. Low power for HL, Larynx, LL, SNC **SUMMARY:** **HL, Larynx, LL, SNC: LOW ↓** (Low sensitivity potential bias ↓)

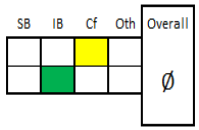
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	All cancer PMR = 1.04.	Co-exposures may have included: phenol, methyl alcohol, glutaraldehyde, mercury, arsenic, zinc, and **ionizing radiation**.	1925 and ceased in 1980.				**ML: Medium ↓** (Potential bias ↓)
Wesseling et al. (1996) Costa Rica Cohort study of banana plantation workers.	26,565 male workers on the payrolls of banana companies as reported to the Social Security Administration between 1972 and 1979. Cohort follow-up in the cancer registry from 1981 to 1992. Losses to follow-up and poor record keeping resulted in difficulty in assessing participation	A list of names of workers sterilized by dibromochloropropane was used to identify banana plantations whose workers may have been exposed to formaldehyde. Co-exposed to maneb, dibromochloropropane, mancozeb, benomyl, chlorothalonil.	Incidence: National Tumor Registry. HL: ICD-9 965-966 MM: ICD-9 973. Higher survival rates for HL and LL could undercount incident cases, but average follow-up is 12 yrs.	Controlled for age and sex. Banana plantation workers are co-exposed to several potential carcinogens such as dibromochloropropane, maneb, mancozeb, benomyl, and chlorothalonil. While these chemical co-exposures are not known risk factors for these outcomes the fact that co-exposures were so high as to	SIR (95% CIs). Latency was not evaluated for these endpoints.	Males: HL: 9 cases MM: 6 cases	 Selection: Selection issues (loss to follow-up, record keeping). Healthy worker effect probable with overall cancer SIR of 0.76. Exposure Group D Possible confounding Very low confidence in data quality **SUMMARY: NOT INFORMATIVE** Critical limitation: (multiple potential

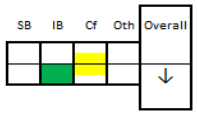
Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	rates. Very low confidence in data quality. Average follow-up ≈11.83 yrs. All cancer SIR = 0.76 (men).			cause sterility in workers strongly suggests a large potential for confounding.			biases and uncertainties)

Table A-106. Evaluation of case-control studies of formaldehyde and cancers of the URT (NPC, SN, OHPC) and LHP (HL, MM, LL, ML)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
Armstrong et al. (2000) Malaysia Population-based case-control study of NPC.	Prevalent and incident NPC cases (31% female) during 1987–1992 identified through treatment or diagnosis records from 4	Individual-level exposure status based on occupational history obtained by interview including job description, worked performed, calendar time, machines, tools, substances used, and exposures to dusts, smoke,	Prevalent and incident cases. Diagnosis of NPC: confirmed by histological review. All cases were squamous cell carcinomas.	Design controlled for age, sex, Chinese ethnicity, and neighborhood. Analysis adjusted for social class, diet, smoking, and wood dust.	Conditional logistic regression; ORs (95% CI) for each of 22 separate occupational exposures. Latency was evaluated (exposures < 1, 5, 10, 15, and 20	NPC: 282 The power to evaluate formaldehyde as a hazard is diminished as fewer than 10% of cases had any exposure to formaldehyde.	 Selection issue with substantial difference in participation rates. Exposure Group B Lack of latency data.

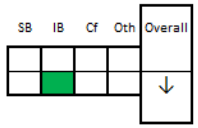
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	radiotherapy centers. Participation of cases was 53% due to death and illness and difficulty in locating them. Participation of living cases who could be located was 89% (*n*=282) and 90% for eligible controls (*n*=282). Selection bias possible. Cases and controls were matched on age, sex, Chinese ethnicity, and neighborhood.	gases, and chemicals. Exposure assessment blinded to outcome.		Other exposures evaluated were wood dust, industrial heat, textile dusts, metals, acids, bases, solvents, detergents, and soaps. Wood dust is a potential confounder but was controlled for.	yrs prior to diagnosis). 8/564 subjects (1.4%) had more than 10 yrs of potential exposure outside of a 10-yr latency period. This suggests additional information bias.		Very low power to detect any effects beyond a 10-yr period. **SUMMARY: NOT INFORMATIVE** (multiple potential biases ↓ and uncertainties)

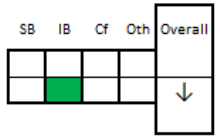
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	Participation rate was somewhat lower in more affluent neighborhoods (80% vs. 90%).						
Berrino et al. (2003) Europe Population-based case-control study of larynx and hypopharynx cancer.	Male residential populations of 6 cancer registries in 4 European countries during 1979–1982. All patients with newly diagnosed cancer were identified with participation rates of 70% to 92% by center. Controls participated at an average rate of 74%.	Individual-level exposure status based on lifetime occupational history for all jobs held for more than 1 yr obtained from questionnaire including job title, specific tasks, and calendar time. Multiple exposure metrics including peak, average, and cumulative exposure developed by job exposure matrix. However, the quality of the exposure assessment is further degraded by	Incident cases. Diagnosis of cancer of the larynx or hypopharynx confirmed by pathology review. Cancer of the larynx divided into epilarynx and endolarynx. Analyses of hypopharynx grouped together with epilarynx while endolarynx analyzed separately. No separate analysis of hypopharynx without epilarynx.	Controlled for age and sex by selecting controls from stratified population samples. Analysis controlled for study center, age, tobacco smoking, socioeconomic status, alcohol, and diet. Exposures to other compounds were identified and evaluated as risk factors including	Unconditional logistic regression; OR (95% CI). Lagged exposures were evaluated to account for cancer latency in selected analyses.	Larynx (endolarynx): 213 total cases 37 cases exposed at least 10 yrs and more than 20 yrs since first exposure.	 Exposure Group B downgraded to Group D based on poor performance of JEM. Confounding likely due to collinearity of exposures to other risk factors and potentially poor quality exposure data which minimized ability to control. **SUMMARY: NOT INFORMATIVE** Critical limitation: Confounding

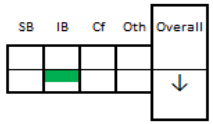
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	Controls were selected from age and sex stratified random samples of the local general population.	the authors' statements. Namely, the authors regarded the "JEM performance as poor for formaldehyde where 14% of jobs classified as category 1 (unexposed) by the matrix were judged as definitely exposed by the experts." Co-linearity among crude exposures (e.g., solvents and formaldehyde had Spearman correlation of 0.4).		asbestos, arsenic, solvents, and dusts (wood and other). Note that solvents were a stronger risk factor for laryngeal cancer than formaldehyde (OR=2.21 vs. 1.7). Co-exposures were controlled for but poorly measured covariates cannot be well controlled for.			
Blair et al. (2001) United States Population-based case control of leukemia.	White men, ages ≥ 30 years. Cases (*n*=513) identified 1980-1983 (cancer registry and hospital network).	Individual-level exposure status based on lifetime farm and nonfarm occupational history for all jobs held for more than one year obtained from interview including job title,	Incident cases. Diagnosis of myeloid leukemia and lymphatic leukemia confirmed by pathology review.	Analysis controlled for age, state, direct or surrogate interview, and smoking. Other co-exposures were not evaluated as	Logistic regression; ORs (95% CI) by exposure categories (3 levels) for intensity, probability, duration, and time since first	ML: 22/59 exposed (14 acute; 8 chronic) LL: 30/190 exposed	 Exposure Group C Lack of latency analysis Possible confounding although relationship

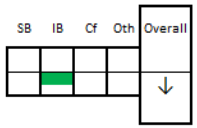
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	Controls (*n*=1,087) selected by random digit dialing (under age 65) otherwise from lists provided by the HCFA and state death files. Controls were frequency-matched by 5-yr age groups, vital status at interview, and state of residence. Cases participation rate was 86%. Control participation rate was 77-79%.	industry, and calendar time. Other exposures evaluated included benzene, other organic solvents, petroleum-based oils & greases, cooking oils, ionizing radiation, paper dusts, gasoline and exhaust vapors, paints, metals, wood dust, asbestos, asphalt, cattle, meat, solder fumes.		potential confounders.	exposure measures. Latency not evaluated.		between formaldehyde and co-exposures is unknown. **SUMMARY:** LM: LOW ↓ (Potential bias ↓)

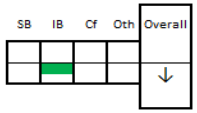
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
D'Errico et al. (2009) Italy Hospital-based case-control study of SNC in the Piedmont region of Italy.	154 sinonasal cases during 1996–2000 identified through treatment or diagnosis records from all Piedmont hospital departments. 5 cases excluded (3 prevalent cases, 2 <30 yrs old). Participation of incident cases using full questionnaire was 76% (113/149). Participation of eligible hospital controls (*n*=336) was 95%.	Lifetime job history (all jobs); company, job title, tasks, size of work environment, and other details. Probability of exposure was determined by blinded expert staff for jobs lasting 6 or more mos. Other exposures evaluated were arsenic, wood dust, leather dust, nickel, chromium, PAHs, welding fumes, oil mists, flour dust, cocoa powder, silica, coal dust, textile dusts, acid mists, paint mists, organic solvents.	Incident cases by cell type were taken from the regional Sinonasal Cancer Registry reported to them by hospitals in the region.	Analysis controlled for age, sex, province of residence, smoking and co-exposures. Wood dust is a considered an extremely strong risk factor for SNC and a potential confounder and was controlled for but adjusted results not presented; just "loss of statistical significance."	Unconditional logistic models; ORs (95% CI). Latency was evaluated with a 10-yr latency period.	SNC: 7/113 exposed The power to evaluate formaldehyde as a hazard is diminished as fewer than 10% of cases had any exposure to formaldehyde.	 Exposure Group B Wood dust is a likely confounder and no effect estimate adjusted for wood dust was presented. Low power **SUMMARY: NOT INFORMATIVE** Critical limitation: Confounding

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	Controls frequency matched for age, sex, and province of residence.						
Gérin et al. (1989) Canada Population-based case-control study. Related study: Siemiatycki et al. (1987)	3,726 male cases, 1979–1985, from 14 major area hospitals, which report to the Quebec Tumor Registry (97% of all cancers reported). 533 population controls participated out of 740 selected (72%). Interviews and questionnaires completed for 82% of eligible cases	Lifetime job history included company activities, raw materials and final product, machines, tasks involving machine maintenance, type of room. A team of chemists and hygienists (likely blinded to outcome) translated each job into a list of potential formaldehyde exposures based on their confidence level, the frequency, and the duration of exposure.	Incident cases histologically confirmed diagnosis of Hodgkin lymphoma (ICD: 201).	Controlled for age, ethnic group, socio-economic status, smoking, and dirtiness of jobs held (white vs. blue collar). Additional control for any of 300 of the most common occupational exposures if the inclusion changed the formaldehyde OR by more than 10%.	Logistic regression; OR (95% CI). Latency not evaluated.	HL: 8/53 exposed.	 Exposure Group B Lack of latency analysis. **SUMMARY:** HL: MEDIUM ↓ (Potential bias ↓).

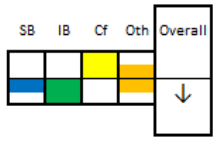
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	of which 18% of interviews were completed by next of kin. Internal and external comparison. Controls were patients with cancer at other sites with all lung cancers excluded. External comparison with general population.						
Heineman et al. (1992). Denmark. Cancer registry-based case-control study, MM diagnosed 1970–1984.	2,098 men registered in both the national cancer registry and pension fund. All men with a specific occupational	Individual-level exposure estimated by industrial hygienists based on occupation listed on most recent tax documents.	Incident cases identified in Danish Cancer Registry. 92% of cases were histologically confirmed.	Controlled for age and gender. Other compounds were identified and evaluated as independent risk factors including:	Logistic regression, ORs (95% CI) by likelihood of exposure in 3 categories. Latency not evaluated.	MM: 835 (185 exposed).	 Exposure Group D Latency not evaluated. Confounding unlikely.

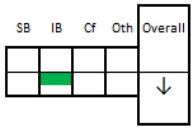
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	history were included. Controls frequency matched on age, sex, and year of diagnosis.			gasoline, oil products, engine exhausts, benzene, dyes, phthalates, vinyl chloride, asbestos, and pesticides. Asbestos is not a risk factor for LHP. 'Possible' benzene exposure was associated with MM but not 'probable' Benzene exposure, so confounding is considered to be unlikely.			SUMMARY: MM: LOW ↓ (Potential bias ↓).
Hildesheim et al. (2001). Taiwan. Population-based case-control study. Related studies:	375 men and women with NPC and 375 controls. Ages <75 yrs, July 1991 and January 1995,	Lifetime job history (jobs held for at least one year since age 16); job title, typical activities/duties, type of industry,	Incident cases. Diagnosis of nasopharyngeal was confirmed by histological review with >90% diagnosed with nonkeratinizing and	Adjusted for age, sex, education, ethnicity, and HLA. Did not adjust for residence.	Logistic regression; ORs (95% CI) by exposure intensity, exposure probability, cumulative	NPC: 375 cases (74 ever exposed)	 Exposure Group B The impact of not controlling for all

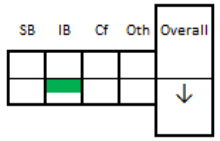
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
Yang et al. (2005); Cheng et al. (1999); Hildesheim et al. (1997)	from 2 hospitals. Participation of eligible cases was 99% and 87% for controls. Controls individually matched 1:1 on age, sex, and district/township of residence.	and tools and/or materials used. Industrial hygienist assigned scaling to subjects based upon intensity and probability of exposure on a scale from 0–9.	undifferentiated carcinomas and 9% with squamous cell carcinoma.	Other exposures identified included: wood dust, solvents, and smoking. All subjects were tested for EBV. The observed associations were not materially affected when controlling for wood dust, smoking and solvent exposure.	exposure and an induction period of 10 yrs used to account for latency. Conditional logistic regression was not used; however, logistic regression did control for age and sex. Area of residence was expected to be related to referral patterns and may not be related to exposure independent of occupational history.		matching factors is unclear. SUMMARY: NPC: MEDIUM ↓ (Potential bias ↓)
Laforest et al. (2000) France Hospital-based case-control study of hypopharyngeal and laryngeal cancer.	Male cases (201 primary hypopharyngeal squamous cell cancer, 296 laryngeal cancer), diagnosed	Occupational histories from questionnaires; industry and occupation coding used with job exposure matrix for	Incident cases. Diagnosis of hypopharyngeal and laryngeal cancers was histologically confirmed.	Controlled for sex, age, alcohol, and smoking. Induction periods of 5, 10, and 15 yrs was also used to	Unconditional logistic regression; OR (95% CI). Latency was evaluated.	OHPC: 201 Larynx: 296	 Exposure Group C SUMMARY: OHPC: MEDIUM ↓

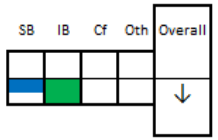
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	during 1989–1991, from 15 French hospitals. Interviews completed for 79.5% of eligible cases and 86% of eligible controls. Controls frequency matched on sex, age, and the same or similar nearby hospital.	formaldehyde (and other exposures). Exposure assessment based on job-exposure matrix that included level and probability of exposure to formaldehyde as well as duration and cumulative exposure to formaldehyde.		account for latency in evaluating risk. Other exposures evaluated included: coal dust, leather dust, wood dust, flour dust, silica, and textile dust. Of these, only coal dust significantly increased the risk of hypopharyngeal cancer in this study but coal dust was controlled for in the OHPC analysis.			(Potential bias ↓)
Luce et al. (2002) China, France, Germany, Italy, Sweden, United States Leclerc et al. (1994); Luce et al. (1993); Magnani	Pooled analysis of 12 case-control studies. Men and women. All from 7 different countries	Occupational histories from interview or questionnaires; industry and occupation coding used with job exposure matrix for	Diagnoses originally assessed in 12 studies. 195 cases were adenocarcinomas (169 men and 26 women) and 432 were squamous cell	Adenocarcinoma results in men controlled for age, study, and cumulative exposure to wood and leather dust. All	Unconditional logistic regression; OR (95% CI). Latency evaluated.	SNC: 627 cases (135 adenocarcinomas exposed. 132 squamous cell carcinomas exposed)	 Exposure Group C SUMMARY:

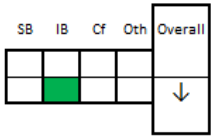
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
et al. (1993); Comba et al. (1992a); Comba et al. (1992b); Luce et al. (1992); Zheng et al. (1992); Vaughan and Davis (1991); Bolm-Audorff et al. (1990); Vaughan (1989); Hayes et al. (1986b); Hayes et al. (1986a); Merler et al. (1986); Vaughan et al. (1986a, 1986b); Hardell et al. (1982) Mack and Preston-Martin (unpub. data) Brinton et al. (1985); Brinton et al. (1984)	diagnosed with sinonasal cancer during 1968–1990. Each individual study selected controls intended to be comparable to the cases in that study.	formaldehyde (and other exposures).	carcinomas (330 men and 102 women).	other results adjusted for age and study. Co-exposures were evaluated as potential confounders. Other occupational exposures potentially affecting risk estimates were controlled for including dusts (wood, leather, coal, flour, textile), silica, asbestos, and man-made vitreous fibers.			SNC: MEDIUM ↓ (Potential bias ↓)

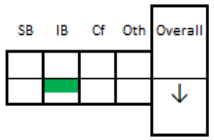
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
Mayr et al. (2010) Germany Hospital-based case-control study.	Hospital patients diagnosed at the University of Erlangen-Nuremburg, Germany during 1973–2007. 31 of 58 patients with identified adenocarcinoma (53%) were followed up with a standardized questionnaire. 85 of 110 patients with cancer of the oral cavity (77%) included as controls. Controls were other hospital patients diagnosed with oral	Structured interview with specific questions about exposure to formaldehyde (and other exposures). Both cases and controls were blinded to case status and study hypotheses, and were not aware of their "case" status.	Prevalent cases. Diagnosis of sinonasal adenocarcinoma in the Department of Otolaryngology, Head and Neck Surgery.	Controlled for age and sex. Other exposures: Wood dust, preservatives, stains, varnishes, solvents, and pickling solutions. Wood dust is a considered an extremely strong risk factor for SNC was not controlled for so there is a strong possibility of confounding.	Crude ORs (95% CI). Methods unstated for OR determinations. Latency not evaluated.	SNC: 2/31 exposed Low power due to the rarity of cases.	 Potential selection issue (prevalent cases) Exposure Group C Latency not evaluated Wood dust is a likely confounder. SUMMARY: NOT INFORMATIVE Critical limitation: Confounding

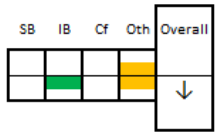
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	cancer during the same time period as cases and in the same hospital. Oral cancer could be related to formaldehyde exposure but this would bias towards the null.						
Olsen and Asnaes (1986b) Denmark Cancer registry-based case-control study, SNC diagnosed 1970-1982. Related study: Olsen et al. (1984)	310 men with incident SN cancer. 215 (69%) squamous cell & lymphoepithelioma. 39 (13%) adenocarcinoma. 2,465 controls, selected among people with colon,	Employment histories from 1964 based on linkage to population registry data; includes industry and job title. Occupational exposure to formaldehyde estimated by industrial hygienists based on industry or occupations.	Incident cases identified in Danish Cancer Registry. Cancer of the nasal cavity (ICD-7 160.0) or sinuses (ICD-7 160.2–160.9) was histologically confirmed. Of all male cases for cancer of the nasal cavity and paranasal sinuses. 82% were squamous cell, lymphoepithelioma	Matched for age, sex, and year of diagnosis. Mantel-Haenszel summary estimates of the relative risk were used to account for possible confounding because the subjects were stratified according to several variables.	OR (95% CI) calculated using the method of Rothman and Boice (1979). Latency was evaluated.	SNC: 215 squamous cell and lymphoepitheliomas (13 exposed to formaldehyde) and 39 adenocarcinomas (17 exposed to formaldehyde)	 Exposure Group C SUMMARY: SNC: MEDIUM ↓ (Potential bias ↓)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	rectum, prostate, and breast cancer diagnosed during the same time period as cases. Controls were selected to be similar with regard to age, sex, and year of diagnosis.		18% were other types.	Wood dust is a considered an extremely strong risk factor for SNC so exposure to wood dust was evaluated as a potential confounder and as an effect modifier.			
Olsen et al. (1984) Denmark Cancer registry-based case-control study, NPC diagnosed 1970-1982. Related study: Olsen and Asnaes (1986b)	266 incident NPC and 488 incident SN cases; matched approximately 3 controls per case. Controls matched on age, sex, and year of diagnosis from the Registry.	Employment histories from 1964 based on linkage to population registry data; includes industry and job title. Occupational exposure to formaldehyde estimated by industrial hygienists based on industry or occupations. Authors reported that 4.2% of control males and 0.1% of females were	Incident cases identified in Danish Cancer Registry. NPC: ICD 146 SN: ICD 160.0 and 160.2–160.9 9% of NPC and SNC cases were sarcomas and 91% were carcinomas. Sarcomas were excluded but gender-specific case counts were not provided for carcinomas.	Controlled for age, sex, and year of diagnosis from the registry. Other exposure evaluated included: wood dust, paint, lacquer, and glue. Wood dust is associated with SNC and was evaluated as a	OR (95% CI) calculated using programs developed by Rothman and Boice (1979). Latency was evaluated.	NPC: 266 cases (number exposed is not stated) SNC: cases included in Olsen and Asnaes (1986a).	 Exposure Group C SUMMARY: NPC: MEDIUM ↓ (Potential bias ↓)

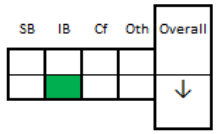
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
		exposed to formaldehyde.		potential confounder of NPC but was not a risk factor.			
Pesch et al. (2008) Germany Insurance-based case-control study.	Male workers insured by a liability insurance association for the German wood-working industries with an occupational disease during 1994–2003. 86/129 cases (67%) participated (including 29 next of kin). 204/272 controls (75%) participated (including 69 next of kin).	Lifetime job history, with focus on tasks and exposures in wood industries. Because next-of-kin information on exposure to wood additives was considered poor, the probability of exposure to formaldehyde was rated by an expert team as none, low, medium, or high.	Prevalent cases. Cases were ever employed in German wood industries and diagnosed with histopathologically confirmed sinonasal adenocarcinoma. Because cases and controls were stratified by age less than 60 yrs and greater or equal to 60 yrs, the older cases may have been selected for survival. If so, this may have resulted in a downward bias.	Controlled for age, smoking, region, interviewee, and average wood dust exposure. Co-exposure to wood preservatives, varnishes, and pigment stains likely. Wood dust is a considered an extremely strong risk factor for SNC but was controlled for.	Logistic regression. OR (95% CI). A 5-yr latency period was applied.	SNC: 47/86 cases exposed	 Potential selection issue (prevalent cases) may have resulted in a downward bias. Exposure Group B Latency evaluation likely to be under-powered to detect any effects beyond a 5-yr period. SUMMARY: SNC: LOW ↓ (Potential bias ↓)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	Controls were selected from the same insurance database of workers with registered accidents. Controls were crudely frequency matched on age with a cut-off at 60 yrs. Median ages were both 69 yrs with cases ranging from 41–84 yrs and controls ranging from 37–85 yrs).						
Pottern et al. (1992) Denmark Cancer registry-based study, MM diagnosed during 1970–1994.	363 female incident cases; included if found in pension fund registry.	Individual-level exposure estimated by industrial hygienists based on occupation listed on most recent annual income tax documents and the	Incident cases identified in Danish Cancer Registry. ICD code at time of diagnosis.	Controlled for age, sex, and vital status. Other exposures evaluated included 19 categories	Logistic regression, ORs (95% CI) by likelihood of exposure in 3 categories.	MM: 60/363 exposed	 Exposure Group D Latency not evaluated

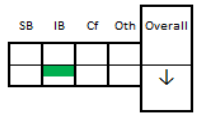
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	1,517 age and sex matched controls alive at time of case diagnosis. All women with a specific occupational history other than "Homemaker" were included.	industry associated with that occupation.		grouping 47 substances. Co-exposures were not evaluated for confounding but exposure to organic solvents (including benzene) and radiation were not risk factors for MM.	Latency not evaluated.		SUMMARY: MM: LOW ↓ (Potential bias ↓)
Roush et al. (1987) United States Population-based case-control study.	173 male cases of NPC, 198 male cases of sinonasal cancer identified from the Connecticut Tumor Registry who died during 1935–1975; and 605 male controls dying during	Job history obtained by city directories and death certificates, which yielded information on job, industry, employer, and year of employment. Job data sought for 1, 10, 20, 25, 30, 40, and 50 yrs prior to death. An industrial hygienist, blinded	Incident cases (from state tumor registries) who had died. Diagnosis of nasopharyngeal cancer and sinonasal cancer based on case registration by the Connecticut Tumor Registry. Clinical records reviewed for >75% of cases.	Controlled for age at death, year at death, and availability of occupational information. Exposure to wood dust was not found to be a risk factor for all nasal cancers (NPC+SNC). This suggests a lower potential for	Logistic regression; ORs (95% CI). Intensity of the likelihood of exposure and latency evaluated.	NPC: 21/173 exposed SNC: 21/198 exposed	 Exposure Group C SUMMARY: NPC, SNC: MEDIUM ↓ (Potential bias ↓)

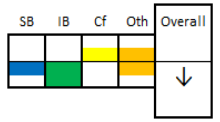
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	the same time period and randomly selected from state death certificates. Controls were matched on sex, date of death, and state of residence.	to case status, classified likely exposure to formaldehyde on basis of job title.	Histological typing not reported.	confounding by wood dust.			
Shangina et al. (2006) Europe Multicenter case-control study.	316 male cases of laryngeal cancer between the ages of 15–79 yrs residing in four European countries that were diagnosed during 1999–2002 and identified by study centers in Romania, Poland, Russia, and Slovakia. 728	Occupational histories obtained by interview and yielded information on all jobs held >1 yr. A general questionnaire obtained information of job titles, tasks, industries, starting and stopping times, full-time/part-time status, working environments, and specific exposures. A specific questionnaire was completed for employment in	Diagnosis of laryngeal cancer was histologically or cytologically confirmed and included topographic subcategories from ICD-O code C32 (glottis, supraglottis, subglottis, laryngeal cartilage, overlapping lesion of the larynx, and larynx, unspecified).	Controlled for age, country, smoking, and alcohol. Other exposures that were found to be risk factors included dusts of "hard alloys" (16 cases) and chlorinated solvents (15 cases). As formaldehyde, hard alloy dust and chlorinated solvents were	Logistic regression; ORs (95% CI). Latency was evaluated.	Larynx: 18/316 exposed The power to evaluate formaldehyde as a hazard is diminished as fewer than 10% of cases had any exposure to formaldehyde.	 Exposure Group C Low power due to rarity of exposure SUMMARY: Larynx: MEDIUM ↓ (Potential bias ↓ low sensitivity)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	male hospital controls selected within 6 mos of case recruitment from diagnoses excluding disease related to alcohol or tobacco. Controls frequency matched by age +/- 3 yrs.	defined jobs or industries.		each found in fewer than 6% of cases, the correlation between them is considered to be small enough to make confounding unlikely.			

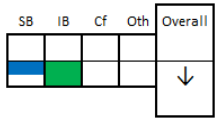
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
Talibov et al. (2014) Europe Multicountry case-control study.	Individuals from Finland, Iceland, Norway, and Sweden who were recorded in various censuses from 1960–1990. Acute myeloid leukemia cases identified by national registries up until 2003–2005 depending on the country.	Occupational history from census records were linked to the Nordic Occupational Cancer Study (NOCCA) JEM to code each cohort member as exposed to formaldehyde. Exposures were quantified based on the proportion of people in each occupation considered to be exposed and the mean level of exposure during specific time periods. 8% of AML cases and controls were exposed. Co-exposures to solvents was evaluated.	Diagnosis of incident cancer reported to the National Cancer Registries.	Controlled for age (<50, 50+), sex, and solvents. Solvents included: aliphatic and alicyclic hydrocarbons, aromatic hydrocarbons, benzene, toluene, trichloroethylene, 111-trichloroethane, methylene chloride, perchloroethylene, other organic solvents, and ionizing radiation.	HRs (95% CI). A 10-yr latency period was assumed.	AML: 1201/15,332 exposed	 Exposure Group D SUMMARY: LOW ↓ (Potential bias ↓)

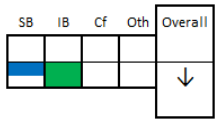
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
Teschke et al. (1997) Canada Population-based case-control study of nasal cancer.	48 incident cases of nasal cancers (31% female) older than 19 yrs, 1990–1992. Controls were randomly selected from age and sex strata of voter list of the same time period. 6 of 54 cases (11%) were excluded for lack of interview as were 36 of 195 controls (18%). Controls matched on age and sex.	Standardized questionnaire including occupational, residential, smoking, and medical histories aimed at identifying exposures considered to be probably carcinogenic by IARC. Occupational data reviewed by an industrial hygienist blinded to case-status. EPA considered that workers in the textile and pulp and paper mill industries may have been exposed to formaldehyde but the exposure questionnaire did not identify them as exposed.	Incident cases from British Columbia Cancer Agency registry. Histologically confirmed primary malignant tumors of the nasal cavity. SNC: ICD-O 160.	Controlled for age and sex. More than 40 specific occupational groups were evaluated without control of confounding. Confounding not evaluated. Potential confounders for these outcomes include chlorophenols, acid mists, dioxin, and perchloroethylene and would likely be positively correlated with formaldehyde exposure. However, on acids mists are associated with URT cancers.	ORs (95% CIs). Latency was evaluated.	SNC: 48 3 cases exposed to pulp and paper mills.	 Exposure Group C Potential confounding for pulp and paper mill workers Low power due to rarity of exposure SUMMARY: SNC: LOW ↓ (Potential bias ↓ low sensitivity)

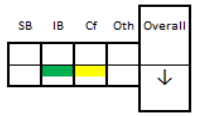
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
		Pulp and paper mill workers may also be co-exposures to dioxin or perchloroethylene (Kauppinen et al., 1997).		Potential for confounding is unknown but could have inflated the observed effect.			
Vaughan et al. (2000) United States Population-based case-control study of nasopharyngeal cancer.	196 cases (32% female) ages 18–74 diagnosed during 1987–1993 identified from 5 population based cancer registries. Interviews completed for 82% of cases and 76% of the 244 controls. 19% of case interviews completed by next of kin.	Individual-level exposure based on industrial hygienist review of detailed occupational histories including industry, job title, duties and dates used to estimate probability, intensity, and cumulative exposure.	Incident cases. Diagnosis of nasopharyngeal (any histological type) based on clinical records. Histological typing reported.	Controlled for age, sex, race, registry, smoking, proxy status, and education. Wood dust evaluated as an independent risk factor for NPC controlling for formaldehyde and it was not a risk factor in this data set. Therefore, wood dust should not be a confounder in this data set.	Logistic regression; ORs (95% CI) by probability of exposure, duration, and cumulative exposure. Separate analyses by histological type. Latency evaluated.	NPC: 79 exposed cases.	 Exposure Group B SUMMARY: NPC: MEDIUM ↓

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	Controls selected by random digit dialing in the same geographical region frequency matched by age, sex, and cancer registry.						
Vaughan (1989) United States Population-based, case control study of squamous cell cancers of the pharynx and sinonasal cavity. Related studies: Vaughan et al. (1986a, 1986b); Included in Luce et al. (2002)	231 cases (32% female) ages 20–74 yrs residing in the area covered by Washington State Cancer Surveillance System during 1980–1983. Participation for all cases was 69% (see see Vaughan et al., 1986a) and 80.0% for	Individual-level exposure based on job exposure matrix by occupation and industry for each individual job used to estimate probability and intensity of exposure. Formaldehyde exposure from available industrial hygiene data, NIOSH and other data, and NCI job exposure linkage system.	Incident cases. Diagnosis of squamous cell cancers of the pharynx and sinonasal cavity based on review of hospital medical records, surveillance of radiotherapy and pathology practices, and state death certificates.	Controlled for age, sex, smoking, and alcohol. NPC analyses controlled for race. Wood dust is associated with URT cancers and would likely be positively correlated with formaldehyde exposure, but strongest association is with SNC.	Logistic regression; ORs (95%CI). Duration of employment and occupation are surrogates for intensity of exposure. Latency was evaluated.	NPC: 3/21 exposed OHPC: 11/183 exposed SNC: cases included in Luce et al. (2002). Low power for NPC and SN.	 Potential selection issue (>40% cases represented by next of kin) Exposure Group D Confounding possible Low power for NPC SUMMARY: NPC: LOW ↓ (Low sensitivity potential bias ↓) OHPC: LOW

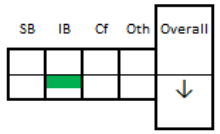
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	controls (*n*=552). ≈50% of cases interviews completed by next of kin. Controls selected by random digit dialing in same residential area as cases and were frequency matched on age and sex with at 2 controls per cases in each 5-year age and sex category. May result in poorer quality exposure data and a bias towards the null.	Occupation as a carpenter or employment in the "lumber and wood product manufacturing" industry presumed to be exposed to formaldehyde.		Potential for confounding is unknown but could have inflated the observed effect.			(Potential bias ↓)

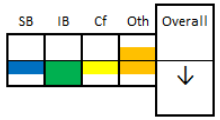
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
Vaughan et al. (1986a) United States Population-based, case control study of cancers (all types) of the pharynx and sinonasal cavity. Related studies: Vaughan (1989); (Vaughan et al., 1986b)@@author-year; SNC cases included in Luce et al. (2002) but not here.	285 cases (35% female) ages 20–74 yrs residing in the area covered by Washington State Cancer Surveillance System during 1980–1983. Participation for all cases was 69% and 80% for controls (*n*=552). ≈50% of cases interviews completed by next of kin. Controls selected by random digit dialing in same residential area as cases and were	Individual-level exposure based on job exposure matrix by occupation and industry for each individual job used to estimate probability and intensity of exposure. Formaldehyde exposure from available industrial hygiene data, NIOSH, and other data, and NCI job exposure linkage system.	Incident cases. Diagnosis of squamous cell cancers of the pharynx and sinonasal cavity based on medical records, surveillance of radiotherapy and pathology practices, and state death certificates. 2% of cases were nonsquamous cell cancers (Vaughan, 1989).	Controlled for age, sex, smoking, and alcohol. NPC analyses controlled for race. Wood dust is associated with risk of URT cancer and was not evaluated as a confounder. However, as this is a case-control study the correlation between formaldehyde and wood dust is expected to be small and thus wood dust would not be expected to be a confounder.	Logistic regression; ORs (95%CI). Latency was evaluated.	NPC: 11/27 occupationally exposed. OHPC: 58/205 occupationally exposed. SNC: cases included in Luce et al. (2002).	 Potential selection issue (>40% cases represented by next of kin) Exposure Group B downgraded to D due to additional measurement error from next-of-kin interviews. Confounding possible for SNC but less so for NPC and OHPC SUMMARY: OHPC, NPC: LOW ↓ (Potential bias ↓)

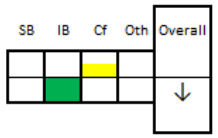
Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	frequency matched on age and sex with at 2 controls per cases in each 5-yr age and sex category.						
Vaughan, 1986, 32316@@author-year} United States Population-based, case control study of cancers (all types) of the pharynx and sinonasal cavity. Related studies: Vaughan (1989); Vaughan et al. (1986a); SNC cases included in Luce et al. (2002) but not here.	285 cases (35% female) ages 20–74 years residing in the area covered by Washington State Cancer Surveillance System during 1980–1983. Participation for all cases was 69% (seeseeVaughan et al., 1986a) and 80% for controls (*n*=552).	Presumed exposure to formaldehyde based on structured telephone interview information on occupational and residential history. Interview-based information on lifetime residential history from cases, next of kin, and controls.	Incident cases. Diagnosis of squamous cell cancers of the pharynx and sinonasal cavity based on medical records, surveillance of radiotherapy and pathology practices, and state death certificates. 2% of cases were nonsquamous cell cancers (Vaughan, 1989).	Controlled for age, sex, smoking, and alcohol. NPC analyses controlled for race. Wood dust is associated with risk of sinonasal cancer and was not evaluated as a confounder. However, as this is a case-control study the correlation between formaldehyde and wood dust is expected to be small and thus	Logistic regression; ORs (95% CI). Latency was evaluated.	NPC: 8/27 lived in mobile home. 10/27 exposed to particleboard. OHPC: 28/205 lived in mobile home. 68/205 exposed to particleboard. SNC: cases included in Luce et al. (2002).	 Potential selection issue (>40% cases represented by next of kin) Exposure Group B downgraded to D due to additional measurement error from next-of-kin interviews. Confounding possible for SNC but less so for NPC and OHPC SUMMARY: OHPC, NPC: LOW ↓ (Potential bias ↓)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	≈50% of cases interviews completed by next of kin. Controls selected by random digit dialing in same residential area as cases and were frequency matched on age and sex with at 2 controls per cases in each 5-yr age and sex category.			wood dust would not be expected to be a confounder.			
West et al. (1993) Philippines Hospital-based case-control study. Related study: Hildesheim et al. (1992)	104 cases (27% female), 11–83 yrs old, predominantly non-Chinese, from the Philippine General Hospital diagnosed before 1992.	Lifetime job history; details not provided. Occupational exposure to formaldehyde classified by blinded industrial hygienist as likely or unlikely to be exposed; appendix provides	Incident cases. Diagnosis of NPC pathologically confirmed by histological review for all cases. Histological typing not reported.	Controlled for age, sex, hospital ward type (or neighborhood), for education, years since first exposure to dust and exhaust fumes, diet including processed meats, fresh fish,	Conditional logistic regression; ORs (95% CI). Latency was evaluated.	NPC: 27/104 exposed	 Exposure Group C Controlling for exposure to mosquito coils which emit formaldehyde may underestimate

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	100% of cases participated. All 104 hospital controls participated while only 77% of 101 community controls participated (Hildesheim et al., 1992). Hospital controls were matched on age, sex, and hospital ward type (private/ public). Community controls were matched on age, sex, and neighbor-hood of residence.	formaldehyde exposure rating for each job category.		smoking, anti-mosquito coils, and herbal medicines. Note that anti-mosquito coils emit formaldehyde 0.87–25 µg/m^3 (Liu et al., 2003). Controlling for mosquito coils may have underestimated to effect of formaldehyde.			the effect of other formaldehyde exposures in the regression analysis. SUMMARY: NPC: MEDIUM ↓ (Potential bias ↓)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
Wortley et al. (1992) United States Population-based, case control study of cancers (all types) of the larynx.	235 cases (21% female) ages 20–74 yrs residing in the area covered by Washington State Cancer Surveillance System during 1983–1987. Participation for all cases was 81% and 80% for controls (*n*=547). 7% of cases interviews completed by next of kin. Controls selected by random digit dialing in same residential area as cases and were	Individual-level exposure based on job exposure matrix by occupation and industry for each individual job used to estimate duration and intensity of exposure. Formaldehyde exposure from available industrial hygiene data, NIOSH, and other data, and NCI job exposure linkage system.	Incident cases. Diagnosis of cancer of the larynx based on medical records, surveillance of radiotherapy and pathology practices, and state death certificates. 94.5% of cases were squamous cell cancers.	Controlled for age, smoking, and alcohol. Further adjustment for sex did not change results. Other exposures: asbestos, chromium, nickel, cutting oils, and diesel fumes. High risk occupations (e.g., mechanics, carpenters, painters, textile machine operators) likely had co-exposures to unidentified substances. However, as this is a case-control study the correlation between formaldehyde	Logistic regression; ORs (95%CI). Latency was evaluated.	Larynx: 58/235 occupationally exposed	 Exposure Group C SUMMARY: Larynx: MEDIUM ↓ (Potential bias ↓)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	frequency matched on age and sex with at 2 controls per cases in each 5-yr age and sex category.			and those potential confounders is expected to be small and thus wood dust would not be expected to be a confounder.			
Yang et al. (2005) Taiwan Family-based case-control study. Related studies: Hildesheim et al. (2001); Cheng et al. (1999); Hildesheim et al. (1997)	502 cases recruited from 265 families with 2 or more NPC cases identified from earlier study (Hildesheim et al., 2001). Additional cases obtained from hospitals that treat NPC. Occupational data available for 65% of cases and 57% of controls.	Lifetime job history (jobs held for at least one year since age 16); job title, typical activities/duties, type of industry, and tools and/or materials used. Exposures coded by industrial hygienist. Exposures in 10 yr preceding diagnosis of interview were excluded. Collected information on cigarette smoking, betel nut consumption, wood and formaldehyde	Original case series were incident cases. Unclear if supplemental cases were incident or prevalent. Diagnosis NPC confirmed by histological review on 502 cases from national tumor registry.	Three analyses (check each and be specific). Family control analysis controlled for family, age, sex, education, and ethnicity. This analysis did not control for partial matching on education, ethnicity, or area of residence. Nor did it control for smoking, betel nut consumption, or wood.	Unconditional logistic regression (95%CI) controlling for age and sex. Lagged exposure partially address latency. Controls used here were originally matched to an earlier set of cases, some of whom were included here.	NPC: 502	 Potential selection issue (>40% cases represented by next of kin) Exposure Group D Negative confounding possible The impact of not controlling for all matching factors is unclear but considered most likely to bias towards the null and inflate confidence intervals.

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	203 cases represented by next of kin (>40%). Cases were matched with 2 groups: First with 1,944 familial controls; and second with 327 population controls.	exposure, and Guangdong and other salted fish consumption during childhood.		In this study, smoking was inversely associated with NPC. Because smoking is positively associated with formaldehyde, there may be negative confounding by smoking in this study.			SUMMARY: NPC: LOW ↓ (Potential bias ↓)
Yu et al. (2004) Hong Kong Mortality odds ratio. Related studies: Ho et al. (2006); EHS Consultants Ltd. (1999)	Men and women. Restaurant workers (*n*=1,225) who died during 1986–1995 and were registered as union members by 4 major Chinese-style restaurant workers' unions in	Occupational history obtained from union records. 415 deceased waiters and 140 deceased waitresses and kitchen workers likely exposed to formaldehyde based on independent studies of air quality in service areas of restaurants. Authors discuss	Mortality: Underlying cause of death from Hong Kong Census and Statistics Department. NPC: ICD-9 147 Histological typing not reported.	MOR with Internal control group adjusted for age at death, sex, year of death, and place of origin. Adjusted for age at death, sex, and year of death for external control group. Most adults (90+ %) are seropositive for EBV and thus it	Logistic regression. Mortality odds ratios (MORs) calculated for waiters and waitresses by internal and external controls and for waiters, length of union membership (a surrogate for duration of exposure).	NPC: 21	 Exposure Group C Latency not evaluated Possible confounding by smoking SUMMARY: NPC: LOW ↓ (Potential bias ↓)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	Hong Kong. Cause of death available for more than 80% of restaurant workers.	sources of exposure. Co-exposures include Epstein-Barr virus (EBV), smoking, salted and preserved foods, and other combustion by-products.		cannot be a confounder. Smoking was evaluated as a potential confounder because 49% of staff smoked compared to 27% of population, but it was insufficient to explain the observed effects. Authors stated that with free fresh food available to workers, the availability of preserved or salted food was unlikely to explain the observed effect.	Latency was not evaluated.		

1

Studies in Animals

Respiratory tract cancer

Similar to other sections, studies were evaluated and assigned the following confidence ratings: High, Medium, or Low Confidence, and "Not Informative" based on expert judgement of each study's methodological details related to predefined criteria within five study feature categories (see Appendix A.5.1). In addition to the general considerations outlined in Appendix A.5.1., criteria specific to evaluating respiratory tract cancer were evaluated (see Table A-107 for specific details). With one exception (noted below), studies of experimental animals exposed for at least subchronic duration (shorter exposure durations were not considered informative to this endpoint, given the robust database), and which performed histopathological evaluations of respiratory tract tissues, were evaluated. As these evaluations consider many of the same studies previously evaluated for inclusion in the noncancer respiratory tract pathology section (see Appendix A.5.5), many parallels exist between both sets of evaluations. While the important considerations across the two sections are generally similar, several notable differences exist. For example, duration of exposure was seen as more important for evaluations of dysplasia and neoplasms, as compared with evaluations of noncancer respiratory tract lesions. Conversely, whereas a substantial emphasis was placed on the characterization of the severity of the lesion for noncancer respiratory tract changes, severity was not considered integral to the identification of cancers and dysplasia. Finally, although most studies of respiratory pathology used paraformaldehyde or freshly prepared formalin as the test article, some studies tested commercial formalin. While co-exposure to methanol is a major confounding factor for systemic endpoints, it is considered to be less of a concern when identifying effects of inhaled formaldehyde on respiratory pathology (see Appendix A.5.5 for discussion). Because of the abundance of animal respiratory pathology studies, only those ranked as having Robust or Adequate exposure quality, and several ranked as having Poor exposure quality studies solely because they tested formalin (see evaluations in Appendix A.5.1), were evaluated for their use in describing the potential for formaldehyde inhalation exposure to cause respiratory tract cancers. Additional considerations that might influence the interpretation of the usefulness of the studies during the hazard synthesis are noted, including limitations such as the use of only one test concentration or concentration that are all too high or too low to provide a spectrum of the possible effects, as well as study strengths such as very large sample sizes or use of good laboratory practices (GLP); however, this information typically did not affect the study evaluation decisions.

Studies are grouped according to exposure duration, and then organized alphabetically by first author. If the conduct of the experimental feature is considered to pose a substantial limitation that is likely to influence the study results, the cell is shaded gray; a "+" is used if potential issues were identified, but these are not expected to have a substantial influence on the interpretation of the experimental results; and a "++" denotes experimental features without limitations that are expected to influence the study results. Specific study details (or lack thereof) which highlight a

limitation or uncertainty in answering each of the experimental feature criteria are noted in the cells. For those experimental features identified as having a substantial limitation likely to influence the study results, the relevant study details leading to this decision are bolded.

Table A-107. Evaluation of controlled inhalation exposure studies examining respiratory tract cancer or dysplasia in animals

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (italicized) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations & statistical analysis[d]**	**Overall confidence rating regarding the use for hazard ID[e]**
Criteria relevant to evaluating the experimental details within each experimental feature category	Exposure quality evaluations (see B.4.1.2) are summarized (++ = "robust"; + = "adequate"; gray box = poor); relevance of the tested exposure levels is discussed in the hazard synthesis-studies without tested exposure <15 mg/m^3 are highlighted	Sample size provides reasonable power to assess endpoint(s) in question (e.g., >20/group desired); species, strain, sex, & age relevant to endpoint; no overt systemic toxicity noted or expected	The study design is appropriate and informative for evaluating respiratory tract cancer or dysplasia, including a sufficient exposure duration and/or appropriate timing of endpoint evaluations to allow for cancer to develop, and a lack of additional modifying variables introduced over the course of the study. GLP-compliant studies are highlighted	The protocols used to assess respiratory tract cancer or dysplasia are sensitive and complete (e.g., multiple tissues and sections examined), discriminating (specific), & biologically sound (reliable); experimenter bias minimized (e.g., slides blinded to evaluator[x])	Statistical methods, group comparisons, & data/variability presentation are appropriate & discerning; mortality data are described	Expert judgement based on conclusions from evaluation of the 5 experimental feature categories
Respiratory Tract Cancers—Chronic						
(Appelman et al., 1988**)** **Rat**	++	+ *Small N (N=10);* Note: randomized	**1-yr duration short to allow for cancer development**	+ *Blinding of slides for evaluation NR*	++	**Medium** [1 yr duration]
(Dalbey, 1982**)** **Hamster**	++ Note: 5 hr/d exposure; days and timing of exposure NR	++	++ Note: single concentration (12.3 mg/m^3) lifetime study	**Blinding of slides for evaluation NR; only 2 nasal sections; limited reporting of**	+ *Locations and specific incidence of lesions and other minor details NR*	**Medium** [Limited sampling, evaluation, and reporting]

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (italicized) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations & statistical analysis[d]**	**Overall confidence rating regarding the use for hazard ID[e]**
				histopathology methods; unclear if dysplasia considered		
(Holmstrom et al., 1989b)	**++** Note: high concentration exposure (15.3 mg/m^3); exposed nocturnally, in contrast to other studies	**+** *Small N (N=15-16); some cannibalism; non-URT tumors ≈50% across groups*	**+** *2/16 animals in formaldehyde group developed emphysema* Note: single concentration (15.3 mg/m^3) 2 yr study	**++** Note: slides blinded	**+** *Locations of lesions and other minor details NR*	**Medium** [Some health issues noted; limited reporting]
(Kamata et al., 1997) **Rat**	**+** *Formalin exposure*, with a methanol control (assumed to be based on levels in formalin) Note: methanol considered unlikely to affect endpoint	**+** *Small N for interim sacrifices (N=2–5)* Note: mortality rate doubled at 18.3 mg/m^3; exposure begun at ≈PND35	**++** Note: 2 yr study	**+** *Blinding of slides for evaluation NR*	**++**	**Medium** [Formalin (with methanol control)]
(Kerns et al., 1983) **Mouse** See also (Battelle, 1982) and (Swenberg et al., 1980b)	**++**	**+** *Survival to 18 mos was <33% in all groups (N is >25)* Note: randomized	**++** Note: data from this study based on a 2 yr GLP study (1982)	**+** *Only three nasal sections evaluated; blinding of slides for evaluation NR*	**+** *Limited reporting of dysplasia findings*	**High** [Note: somewhat limited sampling and high mortality]
(Kerns et al., 1983) **Rat**	**++**	**+** *Viral infection at weeks 52–53*	**++**	**+** *Blinding of slides for evaluation NR*	**+**	**High** [Note: transient viral infection]

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (italicized) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations & statistical analysis[d]**	**Overall confidence rating regarding the use for hazard ID[e]**
See also (Battelle, 1982) and (Swenberg et al., 1980b)		Note: considered unlikely to influence these outcomes; randomized	Note: data from this study based on a 2 yr GLP study (1982)	Note: routine analysis of nasal tissues only	*Limited reporting of dysplasia findings*	
(Monticello et al., 1996**)** **Rat**	**++**	**++** Note: randomized	**++** Note: 2 yr study	**+** *Blinding of slides for evaluation NR* Note: routine analysis of nasal tissues only	**++**	**High**
(Sellakumar et al., 1985**)** **Rat** see also (Albert et al., 1982)	**+** *Air controls direct into chamber, not through apparatus* Note: PFA in paraffin oil (commonly used in bubbler-type units); high concentration exposure (18.2 mg/m^3)	**++**	**++** Note: single concentration (18.2 mg/m^3) lifetime study	**+** *Blinding of slides for evaluation not specified*	**++**	**High**
(Woutersen et al., 1989**)** **Rat**	**++**	**++** Note: randomized	**++** Note: 2 yr study	**+** *Blinding of slides for evaluation NR;* Note: routine analysis of nasal tissues only	**++**	**High**
Respiratory Tract Cancers—Subchronic (note: includes 1 study with only 8 weeks of exposure in genetically modified mice)						

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (italicized) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations & statistical analysis[d]**	**Overall confidence rating regarding the use for hazard ID[e]**
(Andersen et al., 2010) **Rat**	**+** *Analytic concentrations NR*	**Small *N* (*N*=8)** Note: randomized	**13 wk duration with no follow up to allow for cancer**	**+** *Blinding NR; limited reporting of slide selection, analysis methods, and number of slides evaluated*	**+**	**Low** [Short duration; small sample]
(Arican et al., 2009) **Rat**	**Analytical method and concentrations NR**	**+** *Small N (N=10)* Note: randomized	**12 wk duration with no follow up to allow for cancer**	**Blinding NR; slide selection, analysis methods, and number of slides or regions evaluated NR**	**+** *Qualitative descriptions only*	**Not informative [short duration; exposure and outcome methods lacking]**
(Casanova et al., 1994) **Rat**	**++**	**Small *N* (*N*=3)** Note: randomized	**12 wk duration with no follow up to allow for cancer**	**Blinding NR; slide selection, analysis methods, and number of slides or regions evaluated NR**	**+** *Qualitative descriptions only*	**Not informative [short duration; small N; outcome methods lacking]**
(Coon et al., 1970) **Dogs**	**++**	**Small *N* (*N*=2); limited reporting (e.g., age, weight, health status, etc.)**	**Multiple species housed and exposed simultaneously; continuous exposure (>22 hr/d); 90d study does not allow for cancer to develop** Notes: single concentration (4.6 mg/m^3) study	**Blinding NR; slide selection, analysis methods, and number of slides or regions evaluated NR**	**+** *Qualitative descriptions only*	**Not informative [outcome methods lacking; short duration; group housed for exposure]**

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (italicized) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations & statistical analysis[d]**	**Overall confidence rating regarding the use for hazard ID[e]**
(Coon et al., 1970) **Guinea pig**	++	**NR age or number of male vs female guinea pigs; small *N* (*N*=15); limited reporting (e.g., age, weight, health status, etc.)**	**Multiple species housed and exposed simultaneously; continuous exposure (>22 hr/d); 90 d study does not allow for cancer to develop** Notes: single concentration (4.6 mg/m^3) study	**Blinding NR; slide selection, analysis methods, and number of slides or regions evaluated NR**	**+** *Qualitative descriptions only*	**Not informative [outcome methods lacking; short duration; group housed for exposure]**
(Coon et al., 1970) **Monkey**	++	**Small *N* (*N*=3); limited reporting (e.g., age, weight, health status, etc.)**	**Multiple species housed and exposed simultaneously; continuous exposure (>22 hr/d); 90 d study does not allow for cancer to develop** Notes: single concentration (4.6 mg/m^3) study	**Blinding NR; slide selection, analysis methods, and number of slides or regions evaluated NR**	**+** *Qualitative descriptions only*	**Not informative [outcome methods lacking; short duration; group housed for exposure]**
(Coon et al., 1970) **Rabbit**	++	**Small *N* (*N*=2); limited reporting (e.g., age, weight, health status, etc.)**	**Multiple species housed and exposed simultaneously; continuous exposure (>22 hr/d); 90 d study does not allow for cancer to develop**	**Blinding NR; slide selection, analysis methods, and number of slides or regions evaluated NR**	**+** *Qualitative descriptions only*	**Not informative [outcome methods lacking; short duration; group housed for exposure]**

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (italicized) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations & statistical analysis[d]**	**Overall confidence rating regarding the use for hazard ID[e]**
			Notes: single concentration (4.6 mg/m^3) study			
(Coon et al., 1970) **Rat**	**++**	**NR number of male vs female nor how many of each strain exposed; limited reporting (e.g., age, weight, health status, etc.)**	**Multiple species housed and exposed simultaneously; continuous exposure (>22 hr/d); 90 d study does not allow for cancer to develop** Notes: single concentration (4.6 mg/m^3) study	**Blinding NR; slide selection, analysis methods, and number of slides or regions evaluated NR**	**+** *Qualitative descriptions only*	**Not informative [outcome methods lacking; short duration; group housed for exposure]**
(Feron et al., 1988) **Rat**	**++** Note: high concentration exposure (> 12 mg/m^3)	**++**	**+** *13 wk duration, but long-term follow up to allow for cancer to develop*	**+** *Blinding NR; limited reporting of analysis methods*	**+** *Limited information (deaths only) to inform timing of tumor development*	**Medium** [Short duration of exposure; limited reporting]
(Horton et al., 1963) **Mouse**	**+** *Analytic concentrations NR* Note: excessive exposure level (≈200 mg/m^3)	**+** *Limited reporting (e.g., age, weight, health status, etc.); high mortality*	**35 wk duration with no follow up to allow for cancer; exposure paradigm of 1 hr/wk considered less informative**	**Nasal tissue not examined; blinding NR; limited reporting**	**+**	**Not informative** [Primary target tissue not examined; study design limited]
(Maronpot et al., 1986)	**Formalin, methanol concentrations NR, and no controls**	**+** *Small N (N=10)*	**13 wk duration with no follow up to allow for cancer**	**+**	**++**	**Low** [Formalin; small sample]

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (italicized) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations & statistical analysis[d]**	**Overall confidence rating regarding the use for hazard ID[e]**
Mouse		Note: randomized		*Blinding NR; limited reporting of analysis methods*		
National Toxicology Program (2017) Mouse	**+** *Analytic concentrations NR*	**++** Note: "randomly assigned"; Males only; ≈25 mice/ group; genetically modified (p53+/-)	**8 wk exposure duration;** *follow up for 32 wk* Note: although unclear if exposure or follow up duration was adequate, the study employed maximally tolerated cumulative dose	**+** *Blinding NR; examined 3 nasal cavity sections (and 1 larynx)* Note: 4 additional pathologists reviewed all tumor slides	**++**	**Low** [very short (8 wk) exposure duration and limited follow up (32 wk) for cancer development]
(Rusch et al., 1983) Rat	**++** Note: test article was not stabilized (negligible methanol) formaldehyde; concentration <3.6 mg/m^3	**++**	**26 wk duration with no follow up to allow for cancer**	**+** *Blinding NR; limited reporting of analysis methods*	**++**	**Low** [Short duration of exposure with no follow up]
(Rusch et al., 1983) Monkey	**++** Note: test article was not stabilized (negligible methanol) formaldehyde; concentration <3.6 mg/m^3	**++**	**26 wk duration with no follow up to allow for cancer**	**+** *Blinding NR; limited reporting of analysis methods*	**++**	**Low** [Short duration of exposure with no follow up]
(Rusch et al., 1983) Hamster	**++** Note: test article was not stabilized	**++**	**26 wk duration with no follow up to allow for cancer**	**+**	**++**	**Low**

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (italicized) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects[a]**	**Study design[b]**	**Endpoint evaluation[c]**	**Data considerations & statistical analysis[d]**	**Overall confidence rating regarding the use for hazard ID[e]**
	(negligible methanol) formaldehyde; concentration <3.6 mg/m^3			*Blinding NR; limited reporting of analysis methods*		[Short duration of exposure with no follow up]
(Wilmer et al., 1989) **Rat**	**+** *Analytic concentrations NR* Note: concentration tested <5 mg/m^3	**++** Note: randomized	**13 wk duration with no follow up to allow for cancer**	**+** *Blinding NR*	**++**	**Low** [Short duration of exposure with no follow up]
(Woutersen et al., 1987) **Rat**	**++**	**+** *Small N (N=10)* Note: randomized	**13 wk duration with no follow up to allow for cancer**	**+** *Blinding NR*	**++**	**Low** [Short duration of exposure with no follow up]
(Zwart et al., 1988) **Rat**	**++** Note: concentration <3.6 mg/m^3	**++**	**13 wk duration with no follow up to allow for cancer**	**+** *Blinding NR*	**+** *Qualitative descriptions only*	**Low** [Short duration of exposure with no follow up]

NR = not reported; N/A = not applicable

[x] Although blinding of slides for evaluation is considered important, it is identified as only a minor limitation for these endpoints, as the pathology is expected to be overt and not reliant on subtle quantitative (e.g., cell counting) or qualitative (e.g., slightly increased proliferation) decisions that would be highly impacted by potential evaluator biases.

[a]Gray = inadequate *N* (*N*= 1 or 2) or multiple less essential study details (e.g., sex, strain) NR; + = inadequate *N* (e.g., *N*= ≥2 to ≤10) or individual less essential study details NR; ++ = adequate *N* (using guidance from OECD TG 452 and TG 413: chronic: ≥20 animals/sex/group; subchronic: 10 animals/sex/group, respectively).

[b]Gray = test protocols for assessing endpoints could not be evaluated or had critical flaws, timing of exposures expected to compromise the integrity of the protocols, protocols completely irrelevant to human exposure; + = informative components of the protocol were NR/insufficiently assessed, limited human relevance or single concentration study; ++ = protocol considered relevant to human exposure.

[c]Gray = uncontrolled variables are expected to confound the results or lack of reporting for lesion incidence and severity; + = limited information provided for observed lesions (i.e., incidence and/or severity) uncontrolled variables may significantly influence results; ++ = adequate reporting of data, no potential confounding identified.

[d]Gray = failure to report a sufficient amount of data to verify results; + = failure to report statistical analyses; ++ = adequate reporting of data.

[e]Designation for the Use for Hazard ID based on EPA judgment and the following criteria: gray = the presence of generally >2 gray boxes in the study feature categories; low = failure in 2 categories; medium = failure in 1 category; high = no category failures; the presence of multiple +'s may demote tier level.

Lymphohematopoietic cancers

Studies examining LHP cancers were evaluated using nearly identical approaches and criteria as those for respiratory cancers (above). One notable difference involved a consideration of the test article as a key component of the review, as co-exposure to methanol in studies using formalin could have a substantial impact on the interpretation of potential LHP cancers (see exposure quality evaluation in Appendix A.5.1). A minor difference involved the preference for microscopic examination of several tissues applicable to assessing potential LHP cancers, and a preference for blinded assessment of the slides.

Table A-108. Evaluation of controlled inhalation exposure studies examining lymphohematopoietic cancers in animals

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (italicized) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation[c]**	**Data considerations & statistical analysis**	**Overall confidence rating regarding the use for hazard ID**
Criteria relevant to evaluating the experimental details within each experimental feature category	Exposure quality evaluations (see B.4.1.2) are summarized (++ = "robust"; + = "adequate"; gray box = poor); relevance of the tested exposure levels is discussed in the hazard synthesis-studies without tested exposure <15 mg/m^3 are highlighted	Sample size provides reasonable power to assess endpoint(s) in question (e.g., >20/group desired); species, strain, sex, & age relevant to endpoint; no overt systemic toxicity noted or expected	The study design is appropriate and informative for evaluating LHP cancer or dysplasia, including a sufficient exposure duration and/or appropriate timing of endpoint evaluations to allow for cancer to develop, and a lack of additional modifying variables introduced over the course of the study. GLP-compliant studies are highlighted	The protocols used to assess LHP cancer or dysplasia are sensitive and complete (e.g., multiple tissues and sections examined), discriminating (specific), & biologically sound (reliable); experimenter bias minimized (e.g., slides blinded to evaluator[x])	Statistical methods, group comparisons, & data/variability presentation are appropriate & discerning; mortality data are described	Expert judgement based on conclusions from evaluation of the 5 experimental feature categories
(Kamata et al., 1997**)** **Rat**	**+** *Formalin exposure*, with a methanol control	**+** *Small N for interim sacrifices (N=2–5);* Note: mortality rate doubled at 18.3 mg/m^3; exposure begun at ≈PND35	**++** Note: 2 yr study	**+** *Blinding of slides for evaluation NR; specific, routine histopathology of several tissues relevant to LHP cancer (e.g., femur)*	**++**	**Medium** [Formalin (with methanol control)]
(Kerns et al., 1983**)** **Mouse**	**++**	**+** *Survival to 18 months was <33% in all groups (N is >25)*	**++** Note: relevant data from the 2-yr GLP study	**+** *Blinding of slides for evaluation NR; reported gross lesions only*	**+** *Limited reporting*	**High** [Note: somewhat limited sampling for potential LHP

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (italicized) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation[c]**	**Data considerations & statistical analysis**	**Overall confidence rating regarding the use for hazard ID**
See also (Battelle, 1982) and (Swenberg et al., 1980b)		Note: randomized	report (1982); (Battelle, 1982)			cancers and high mortality]
(Kerns et al., 1983) Rat See also (Battelle, 1982) and (Swenberg et al., 1980b)	**++**	**+** *Viral infection at weeks 52-53* Note: considered unlikely to influence these outcomes; randomized	**++** Note: relevant data from the 2-yr GLP study report (1982; Battelle, 1982)	**+** *Blinding of slides for evaluation NR; reported gross lesions only*	**+** *Limited reporting*	**High** [Note: transient viral infection; limited sampling for potential LHP cancers]
National Toxicology Program (2017) Mouse	**+** *Analytic concentrations NR*	**++** Note: "randomly assigned"; Males only; ≈25 mice/ group; genetically modified (p53+/-)	**8 wk exposure duration;** *follow up for 32 wk* Note: although unclear if exposure or follow up duration was adequate, the study employed maximally tolerated cumulative dose; however, no increase in any tumors noted (even nasal SCCs, which were the focus of the study hypothesis)	**+** *Blinding NR; slide evaluation details NR, but assessed multiple relevant tissues* Note: 4 additional pathologists reviewed all tumor slides	**++**	**Low** [very short (8 week) exposure duration and limited follow up (32 wk) for cancer development]

	Experimental Feature Categories *The study details leading to identification of major (bolded) or minor (italicized) experimental feature limitations are indicated.*					
	Exposure quality	**Test subjects**	**Study design**	**Endpoint evaluation[c]**	**Data considerations & statistical analysis**	**Overall confidence rating regarding the use for hazard ID**
(Sellakumar et al., 1985) **Rat** see also (Albert et al., 1982)	**+** *Air controls direct into chamber, not through apparatus* Note: PFA in paraffin oil (commonly used in bubbler-type units); high concentration exposure (18.2 mg/m^3)	**++**	**++** Note: single concentration (18.2 mg/m^3) lifetime study	*Does not appear to be an explicit, routine examination of tissues relevant to LHP cancers, or an evaluation of bone marrow, in particular ("histologic sections were prepared from... other organs where gross pathology was present"); Blinding of slides for evaluation not specified*	**++**	**Low** [no routine examination of tissues relevant to LHP cancers, and lack of evaluation of bone marrow specfically, severely limits detection ability]

Supporting Material for Carcinogenicity

Cancer sites for which data were reported that were not formally reviewed in this assessment included lung, non-Hodgkin lymphoma, brain, bladder, colon, pancreas, prostate, and skin cancers. A summary of the studies available on lung, non-Hodgkin lymphoma, and brain are provided below for information. The data on bladder, colon, pancreas, prostate, and skin cancers were sparse and, as such, these studies are not summarized.

Lung Cancer

Evidence describing an association between formaldehyde exposure and the risk of dying from lung cancer is available from 28 epidemiologic studies (Coggon et al., 2014; Beane Freeman et al., 2013; Meyers et al., 2013; Checkoway et al., 2011; De Stefani et al., 2005; Stern, 2003; Marsh et al., 2001; Stellman et al., 1998; Band et al., 1997; Chiazze et al., 1997; Jakobsson et al., 1997; Andjelkovich et al., 1995; Dell and Teta, 1995; Hansen and Olsen, 1995; Hayes et al., 1990; Partanen et al., 1990; Gérin et al., 1989; Solet et al., 1989; Edling et al., 1987b; Robinson et al., 1987; Bertazzi et al., 1986; Bond et al., 1986; Logue et al., 1986; Stroup et al., 1986; Levine et al., 1984a; Liebling et al., 1984; Walrath and Fraumeni, 1984, 1983; Walrath and Jr, 1983). Currently, these are the only primary studies that provide informative evidence of the effect of formaldehyde exposure on the risk of dying from lung cancer. A few studies are interpreted as unlikely to be informative (i.e., i.e., Fryzek et al., 2005; Wesseling et al., 1996; Hansen et al., 1994; Hall et al., 1991; Harrington and Oakes, 1984), based on considerations used to evaluate observational studies in the toxicological review.

Non-Hodgkin Lymphoma

The most specific level of non-Hodgkin lymphoma diagnosis that is commonly reported across the epidemiologic literature has been based on the first three digits of the Eighth or Ninth Revision of the ICD code [i.e., non-Hodgkin lymphoma ICD-8 and ICD-9: Codes 200 and 202 (WHO, 1977, 1967); however, early studies reported results for lymphosarcoma/reticulosarcoma alone (ICD-8/9: Code 200)]. Evidence describing the association between formaldehyde exposure and the specific risk of non-Hodgkin lymphoma was available from 19 epidemiologic studies—four case-control studies (Tranah et al., 2009; Wang et al., 2009b; Blair et al., 1993; Gérin et al., 1989) and 15 cohort studies (Coggon et al., 2014; Meyers et al., 2013; Beane Freeman et al., 2009; Stellman et al., 1998; Band et al., 1997; Andjelkovich et al., 1995; Dell and Teta, 1995; Hansen and Olsen, 1995; Hayes et al., 1990; Matanoski, 1989; Edling et al., 1987b; Robinson et al., 1987; Stroup et al., 1986; Walrath and Fraumeni, 1984, 1983; Walrath and Jr, 1983). One study was interpreted as unlikely to be informative (i.e., i.e., Matanoski, 1989).

Brain Cancer

Evidence describing an association between formaldehyde exposure and the risk of dying from brain cancer is available from 16 epidemiologic studies (Beane Freeman et al., 2013; Meyers

et al., 2013; Hauptmann et al., 2009; Coggon et al., 2003; Stellman et al., 1998; Band et al., 1997; Andjelkovich et al., 1995; Dell and Teta, 1995; Hansen and Olsen, 1995; Hayes et al., 1990; Matanoski, 1989; Robinson et al., 1987; Stroup et al., 1986; Levine et al., 1984a; Walrath and Fraumeni, 1984, 1983; Walrath and Jr, 1983). Currently, these are the only primary studies that provide evidence of the effect of formaldehyde exposure on the risk of dying from brain cancer. A few studies were interpreted as unlikely to be informative (i.e., i.e., Wesseling et al., 1996; Hansen et al., 1994; Hall et al., 1991; Harrington and Oakes, 1984).

Approaches for Cancer Mode of Action

Formal systematic approaches to identifying and evaluating the literature databases of studies examining mechanistic data relevant to interpreting the potential for formaldehyde to cause upper respiratory tract (URT) or lymphohematopoietic (LHP) cancers were not performed. Rather, these sections consider studies identified through other health effect-specific literature searches, and evaluate those studies in the context of the specific cancer etiology being considered. Supplemental literature relevant to interpreting the biological relevance of some mechanistic data was also identified from review articles and other national-level health assessments. These sections rely heavily on searches and evaluations performed in the following sections: genotoxicity, respiratory tract pathology, and integrated noncancer portal of entry mode of action (see Appendices A.4, A.5.5, and A.5.6).

APPENDIX B. INFORMATION IN SUPPORT OF THE DERIVATION OF REFERENCE VALUES AND CANCER RISK ESTIMATES

B.1. DOSE-RESPONSE ANALYSES FOR NONCANCER HEALTH EFFECTS

A thorough understanding of the exposure-response functions for any association between exposure and health outcomes supports both the derivation of the traditional toxicity values (e.g., RfC) as well as potentially allowing for the estimation of risk above and below those values, and thus provides a more comprehensive understanding of the effects of formaldehyde exposure on various health outcomes. The following details on the estimation of points of departure for the derivation of candidate reference concentrations (cRfCs) are provided to support the derivation of toxicity values as well as to directly inform the potential computation of benefits analyses which require detailed information describing the shape of the exposure-response function across a range of exposures. Such benefits analyses may be used to support a variety of rulemakings.

The technical detail on dose-response evaluation and determination of points of departure (POD) for relevant toxicological endpoints are provided in this Section. Some of the endpoints were modeled using the U.S. EPA's Benchmark Dose Software (BMDS, version 2.2). The common practices used in evaluating the model fit and selecting the appropriate model for determining the POD, as outlined in the *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012) were used. For some data, alternative methods were used, and these are noted as necessary in the summary of the modeling results.

B.1.1. Evaluation of Model Fit Using BMDS models

For each dichotomous endpoint, BMDS dichotomous models were fitted to the data using the maximum likelihood method. Each model was tested for goodness-of-fit using a chi-square goodness-of-fit test (χ^2 p-value < 0.10 indicates lack of fit). Other factors were also used to assess model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR.

For each continuous endpoint, BMDS continuous models were fitted to the data using the maximum likelihood method. Model fit was assessed by a series of tests as follows. For each model, first the homogeneity of the variances was tested using a likelihood ratio test (BMDS Test 2). If Test 2 was not rejected (χ^2 p-value ≥ 0.10), the model was fitted to the data assuming constant variance. If Test 2 was rejected (χ^2 p-value < 0.10), the variance was modeled as a power function of the mean, and the variance model was tested for adequacy of fit using a likelihood ratio test (BMDS Test 3). For fitting models using either constant variance or modeled variance, models for the mean

response were tested for adequacy of fit using a likelihood ratio test (BMDS Test 4, with χ^2 p-value < 0.10 indicating inadequate fit). Other factors were also used to assess the model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR.

B.1.2. Noncancer Estimates from Observational Epidemiology Studies

Derivation of BMC and BMCL for Burning Eyes (Hanrahan et al., 1984)

Hanrahan et al. (1984) conducted a cross-sectional study and reported a concentration-response relationship for the prevalence of ocular discomfort (i.e., burning eyes/eye irritation) in a study of 61 teenage and adult residents of mobile homes in Wisconsin during July of 1979. In-home formaldehyde measurements were obtained for all participants, and measured formaldehyde levels (average of two approximately 1-hour air samples—one from the kitchen or living room and one from a bedroom) were used to characterize average in-home exposures.

Hanrahan et al. (1984) reported that prevalent symptoms[24] of burning eyes and eye irritation were significantly associated with in-home formaldehyde exposures, and the authors provided a graphical representation of the best-fitting logistic regression model results of predicted prevalence of "burning eyes" for exposures at 100 ppb increments from 100 to 800 ppb. From inspection of this graph, EPA determined the prevalence of burning eyes predicted at 100 ppb is approximately 4%. While the published exposure-response results were shown truncated at 100 ppb, Hanrahan et al. (1984) reported that exposures ranged from <100 ppb to 800 ppb, and the indoor median formaldehyde concentration was 160 ppb. Based on this information, it is reasonable to assume that there were residential exposures below 100 ppb, and thus the extrapolation of the published results below 100 ppb is considered to be based on measured concentrations within the study's observed exposure range. Thus, it is possible to approximate the functional form of the concentration-response relationship below 100 ppb from the graphical results because what the investigators presented was the model predicted functional form for all measured exposures. The reconstruction of that underlying functional form can show the results of the same Hanrahan et al. (1984) model where they were omitted from the graphic below 100 ppb.

[24]Hanrahan et al. (1984) reported on the "prevalence" of symptoms; however, it is not clear if this was the "point prevalence" of symptoms on the day of the formaldehyde sampling, or whether this was the "period prevalence" of symptoms during the study period (July 1979).

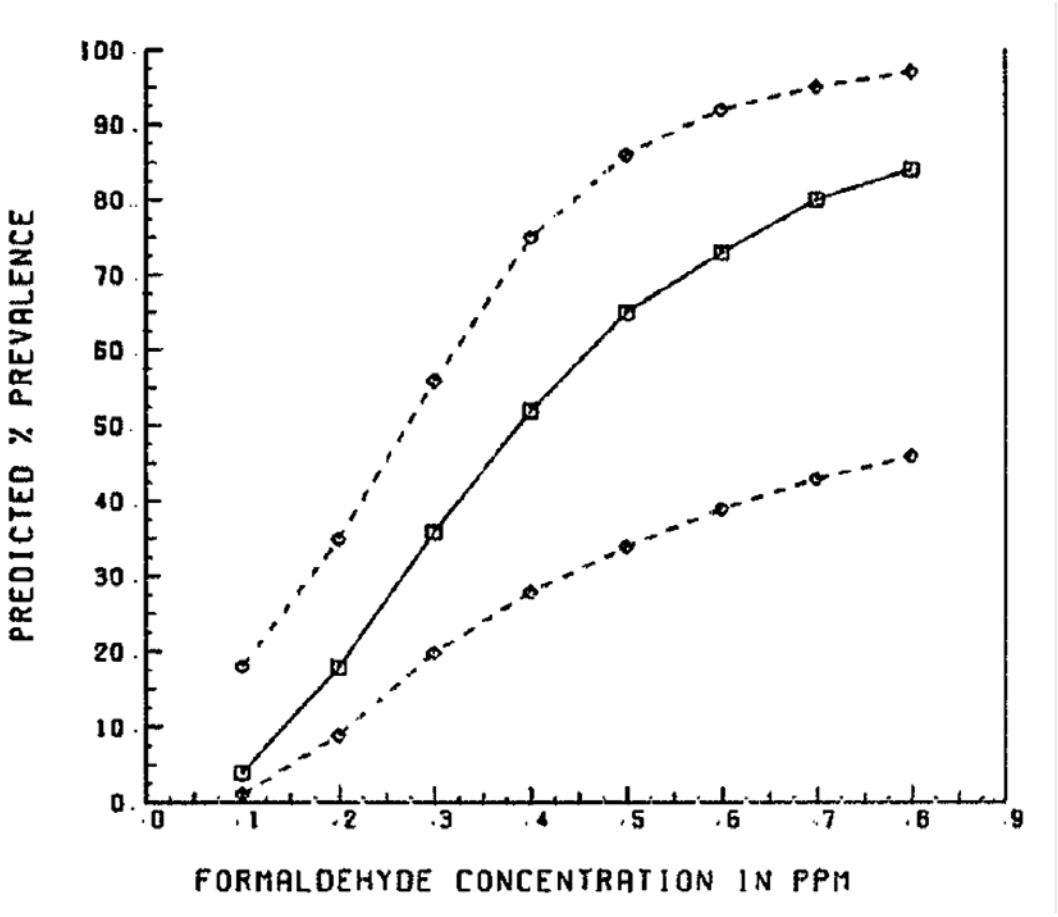


Figure B-1. Regression of prevalence of "burning eyes" versus indoor formaldehyde concentration (ppm) in mobile homes (approximately 1-hour air samples). Dashed lines show upper and lower 95th percentile confidence intervals on model results.

In Figure B-1, the dependent variable is displayed as a predicted percentage prevalence of burning eyes. However, the general epidemiologic method used to model prevalence data is logistic regression, which predicts the log odds of prevalence, which the authors then transformed to prevalence for graphing. In order to describe the underlying functional form of the results displayed, EPA converted the prevalence data back to prevalence odds. Table B-1 shows the prevalence values which EPA visually estimated from the plot, as well as the associated prevalence odds, which EPA calculated as estimated prevalence divided by the complement of estimated prevalence, that is $p/(1-p)$. Figure B-2 plots the estimated prevalence odds against the residential concentration of formaldehyde.

Table B-1. Concentration-response information for the central estimate of the effect extracted from Hanrahan et al. (1984).

Residential formaldehyde concentration (ppm)	Prevalence (p)	Prevalence odds ($p/[1-p]$)
0.1	0.0375	0.039
0.2	0.175	0.212
0.3	0.35	0.538
0.4	0.52	1.08
0.5	0.66	1.86
0.6	0.725	2.64
0.7	0.8	4
0.8	0.85	5.67

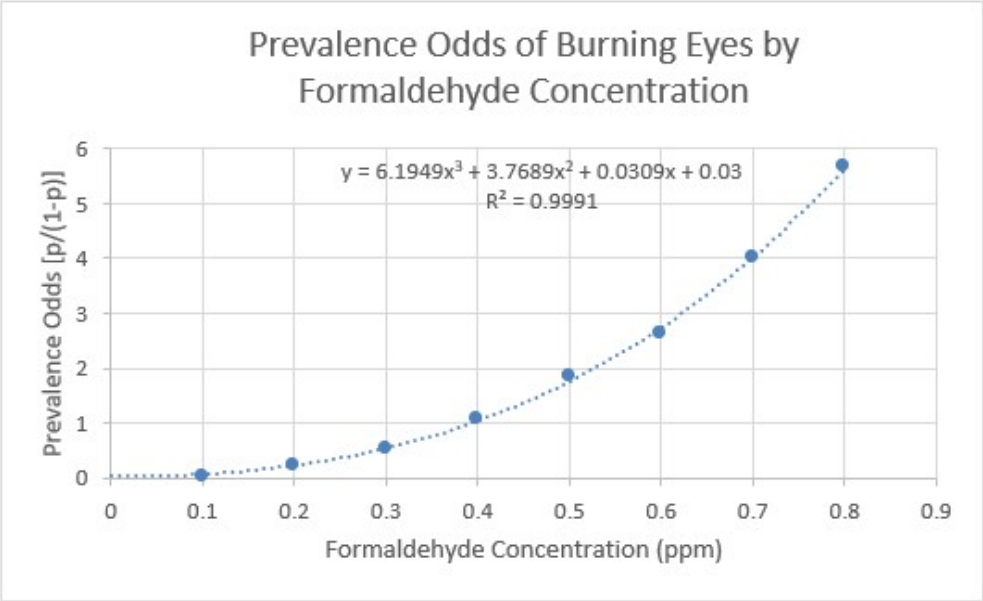


Figure B-2. Plot of the prevalence odds by residential concentration-response information from Table 1.

In order to describe the underlying functional form of the model-predicted results from Hanrahan et al. (1984), EPA fit polynomial trend lines from linear up to cubic functions with the intercept fixed at a background prevalence of burning eyes of 3% [25] (using Microsoft Excel) to the discrete prevalence odds data in Figure B-2 and found that a third degree polynomial function fit

[25]Setting the intercept to other value such as 0.01, 0.02, 0.03 made little difference (e.g., at 0.03, the R^2 had the same value of 0.9991, and the model was $y=6.1949x^3 + 3.7689x^2 + 0.0309x + 0.03$.

with an R^2 value of 0.9991. This indicates nearly a perfect fit to the published model results. Such a high value of R^2 would not have been achieved from analysis of the raw data (unavailable), but the objective here was to recreate the functional form of the modeled data presented by Hanrahan et al. (1984). The following describes the functional form for the prevalence odds:

$$\frac{p}{1-p} = 6.1949 * (exposure)^3 + 3.7689 * (exposure)^2 + 0.0309 * (exposure) + 0.03 \quad \text{(B-1)}$$

Table B-2 shows the prevalence values for the upper bound of the published concentration-response function, which EPA visually estimated from the plot, as well as the associated prevalence odds, which EPA calculated as estimated prevalence divided by the complement of estimated prevalence, that is $p/(1\text{-}p)$. Figure B-3 plots the estimated prevalence odds against the residential concentration of formaldehyde.

Table B-2. Concentration-response information for the upper bound on the central estimate of the effect extracted from Hanrahan et al. (1984)

Residential formaldehyde concentration (ppm)	Prevalence (p)	Prevalence odds ($p/[1\text{-}p]$)
0.1	0.18	0.22
0.2	0.35	0.54
0.3	0.55	1.22
0.4	0.74	2.85
0.5	0.84	5.25
0.6	0.91	10.11
0.7	0.94	15.67
0.8	0.96	24.00

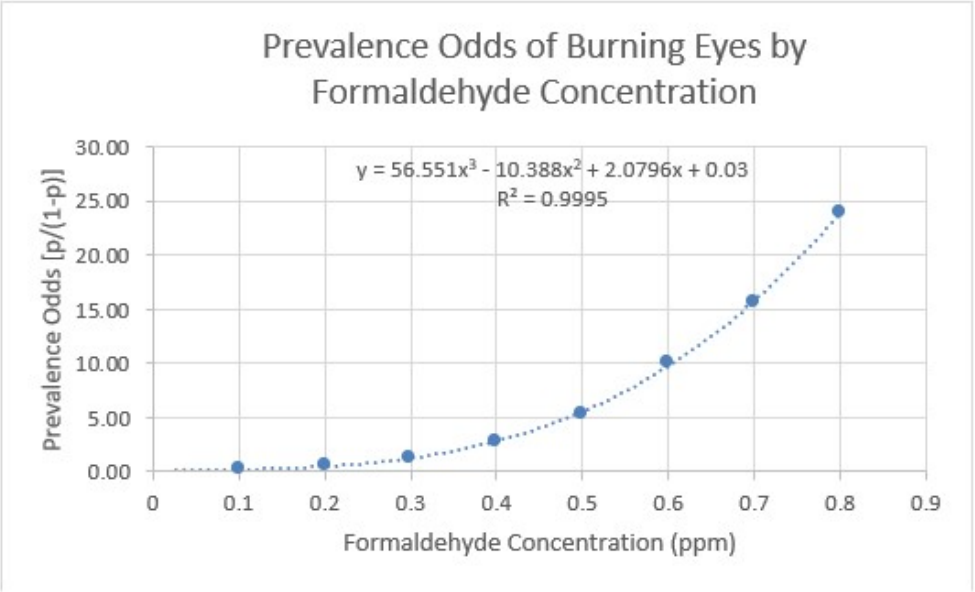


Figure B-3. Plot of the upper bound on prevalence odds by residential concentration-response information from Table 2.

In order to describe the underlying functional form of the model-predicted results from Hanrahan et al. (1984), EPA fit polynomial trend lines from linear up to cubic functions with the intercept fixed at zero (using Microsoft Excel) to the discrete prevalence odds data in Figure 3 and found that a third-degree polynomial function fit with an R^2 value of 0.9995. This indicates nearly a perfect fit to the published model results. The following describes the functional form for the prevalence odds:

$$\frac{p}{1-p} = 56.551 * (exposure)^3 - 10.388 * (exposure)^2 + 2.0796 * (exposure) + 0.03 \qquad \text{(B-2)}$$

Selecting a benchmark response (BMR) for the derivation of a reference concentration (RfC) involves making judgments about the statistical and biological characteristics of the data set. A BMR representing an extra risk of 10% is generally recommended as a standard reporting level for quantal data. Biological considerations may warrant the use of a BMR of 5% or lower for some types of effects (e.g., frank effects), or a BMR greater than 10% (e.g., for early precursor effects) as the basis of the point of departure (POD) for a reference value (U.S. EPA, 2012).

EPA calculated the concentration at which a 10% extra risk of "burning eyes" would have been observed in these data using the polynomial functions for the main effect to estimate the BMC and for the upper-bound to estimate the BMCL. In this derivation, 10% extra risk is the benchmark response (BMR) and the BMC and BMCL for a 10% BMR are noted as the BMC_{10} and $BMCL_{10}$. Note that in Hanrahan et al. (1984), the prevalence of "burning eyes" was similar to that of "eye

irritation." As there is little information available in the literature to estimate the background prevalence of "burning eyes," the background prevalence of "burning eyes" was estimated at 3% (in the absence of formaldehyde exposure) based on the prevalence of "eye irritation." A background prevalence of 3% was considered to be a reasonable estimate. Sensitivity analyses using a background prevalence of 1% and 2% were also evaluated and yielded BMC and BMCL estimates.[26]

Because the extra risk is a function of the prevalence in the exposed ($P_{Exposed}$) and the prevalence in the unexposed ($P_{Unexposed}$) was estimated at 3%, EPA derived $P_{Exposed}$ for 10% extra risk above background.

Extra Risk = 0.10 = $[P_{Exposed} - P_{Unexposed}]/[1 - P_{Unexposed}]$ and $P_{Unexposed} = 0.03$, then $P_{Exposed} = 0.127$.

(B-3)

Because the exposure-response function from Hanrahan et al. (1984) is in terms of the prevalence odds, that value is derived based on $P_{Exposed} = 0.127$. Thus, the prevalence odds = $[P_{Exposed}]/[1-P_{Exposed}] = 0.145$. To derive the BMC, solve for the exposure value, which yields prevalence odds of 0.145:

$$0.145 = 6.1949 * (exposure)^3 + 3.7689 * (exposure)^2 + 0.0309 * (exposure) + 0.03$$

(B-4)

Of the three roots, only one is within the exposure range of the data.

Exposure = 0.153 ppm formaldehyde = 0.188 mg/m^3 formaldehyde (*see footnote*[27])

To derive the interim BMCL, solve for:

$$0.145 = 56.551 * (exposure)^3 - 10.388 * (exposure)^2 + 2.0796 * (exposure) + 0.03$$

(B-5)

Of the three roots, only one is within the exposure range of the data.

Exposure = 0.0706 ppm formaldehyde = 0.0868 mg/m^3 formaldehyde

The BMC_{10} is 0.188 mg/m^3. The $BMCL_{10}$ is 0.0868 mg/m^3.

[26]Using a 1% background prevalence to estimate the exposure-response function and the BMC, yields an estimate of 0.154 ppm = 0.190 mg/m^3 formaldehyde, and a BMCL estimate of 0.0768 = 0.0945 mg/m^3; using a 2% background prevalence to estimate the exposure-response function and the BMC, yields an estimate of 0.154 ppm = 0.189 mg/m^3 formaldehyde, and a BMCL estimate of 0.0739 = 0.0909 mg/m^3.

[27]Concentration (mg/m^3) = Concentration (ppm) * (Molecular mass/Molar volume) = 0.155 ppm * [(30.03 g/mol)/(24.45 L)] = 0.191 mg/m^3 at 25°C.

Eye Irritation Data from Two Controlled Human Exposure Studies (Kulle, 1993; Kulle et al., 1987; Andersen and Molhave, 1983; Andersen, 1979)

Modeling results are presented that support the derivation of PODs for sensory irritation based on two controlled human exposure studies. Kulle et al. (1993) reanalyzed results of a study of eye, nose, and throat irritation among participants exposed to 0, 0.5, 1.0, 2.0, and 3.0 ppm for 3 hours once a week with exposure order randomly assigned. Another experimental study exposed a group of 16 subjects to 0.3, 0.5, 1.0, and 2.0 mg/m^3 formaldehyde for 5-hour periods with a 2-hour clean air exposure prior to each trial (Andersen and Molhave, 1983; Andersen, 1979). The order of exposure concentrations was randomized. The occurrence of irritation symptoms during the clean air exposure was not reported. Two sets of models were evaluated using the data from Andersen (1983; 1979) and estimates of 0% and 3% for prevalence of irritation during the clean air exposure.

Table B-3. Benchmark dose modeling of sensory irritation using a BMR of 10%

Model	BMD	BMDL	AIC	*p*-value	Best model	Notes
Andersen and Molhave, (1983) (Assumed response among controls = 0)						
Gamma	0.209	0.091	58.847	0.0488		
Logistic	0.256	0.182	62.408	0.0665		
Log Logistic	0.257	0.157	57.33	0.1429	X	Lowest AIC
Log Probit	0.249	0.153	57.965	0.1109		
Multistage	0.137	0.068	60.321	0.0161		
Multistage	0.137	0.068	60.321	0.0161		
Probit	0.239	0.175	65.167	0.0469		
Weibull	0.169	-0.077	59.527	0.0404		
Quantal-Linear	0.080	0.060	60.262	0.0247		
Andersen and Molhave, (1983) (Assumed response among controls = 3%)						
Gamma	0.304	0.142	77.217	0.1946		
Logistic	0.201	0.148	76.388	0.0001		
Log Logistic	0.369	0.219	74.821	0.4013	X	Lowest AIC
Log Probit	0.350	0.208	75.8	0.3202		
Multistage	0.262	0.091	79.039	0.1145		
Multistage	0.262	0.091	79.039	0.1145		
Probit	0.196	0.149	77.859	0.0005		
Weibull	0.233	0.108	78.456	0.1696		
Quantal-Linear	0.091	0.065	80.471	0.152		
Kulle et al. (1993)						
Gamma	0.853	0.497	66.839	0.1819		
Logistic	0.760	0.546	64.737	0.3644		
Log Logistic	0.852	0.510	67.596	0.1465		
Log Probit	0.850	0.541	67.254	0.1594		
Multistage	0.676	0.395	65.090	0.3726		
Multistage	0.863	0.369	66.134	0.226		
Probit	0.694	0.502	64.645	0.3686	X	Lowest AIC

Model	BMD	BMDL	AIC	*p*-value	Best model	Notes
Weibull	0.886	0.501	66.225	0.2108		
Quantal-Linear	0.270	0.191	71.876	0.0629		

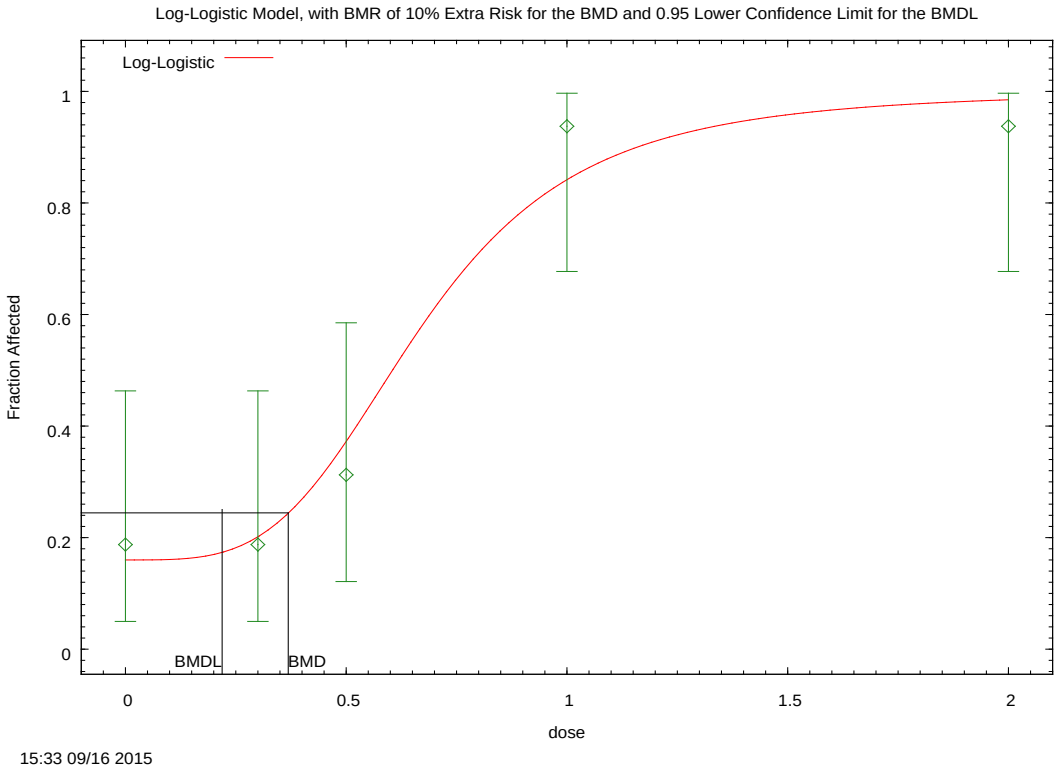


Figure B-4. Log-logistic model with BMC of 10% extra risk over an assumed background of 3% and lower confidence limit for the BMCL for prevalence of conjunctival redness and/or nose or throat dryness; data from Andersen and Molhave (1983).

Table B-4. Parameter estimates for log-logistic model with BMC of 10% extra risk over an assumed background of 3% and lower confidence limit for the BMCL for prevalence of conjunctival redness and/or nose or throat dryness; data from Andersen and Molhave (1983)

Variable	Estimate	Std. err.	Lower conf. limit	Upper conf. limit
Background	0.1604	0.0715851	0.0200953	0.300704
Intercept	1.46207	0.609559	0.267359	2.65679
Slope	3.66848	1.12878	1.45611	5.88085

Table B-5. Observed and estimated values and scaled residuals for log-logistic model with BMC of 10% extra risk over an assumed background of 3% and lower confidence limit for the BMCL for prevalence of conjunctival redness and/or nose or throat dryness; data from Andersen and Molhave (1983)

Dose	Est. Prob.	Expected	Observed	Size	Residual
0	0.1604	2.566	3	16	0.295
0.3	0.202	3.232	3	16	-0.144
0.5	0.3731	5.97	5	16	-0.501
1	0.842	13.472	15	16	1.047
2	0.985	15.76	15	16	-1.561

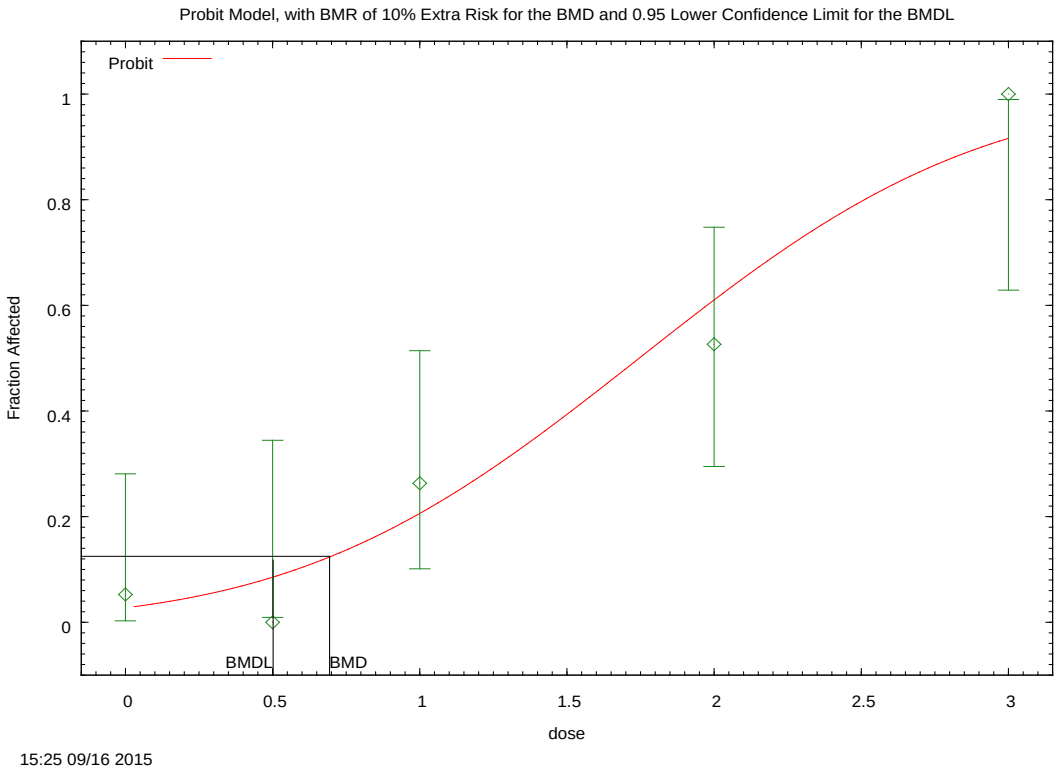


Figure B-5. Probit model with BMC of 10% extra risk and 95% lower confidence limit for the BMCL for prevalence of eye irritation; data from Kulle et al. (1987)

Table B-6. Parameter estimates for probit model with BMC of 10% extra risk and 95% lower confidence limit for the BMCL for prevalence of eye irritation; data from Kulle et al. (1987)

Variable	Estimate	Std. err.	Lower conf. limit	Upper conf. limit
Intercept	-1.9161	0.36123	-2.6241	-1.20811
Slope	1.10331	0.222381	0.667453	1.53917

Table B-7. Observed and estimated values and scaled residuals for probit model with BMC of 10% extra risk and 95% lower confidence limit for the BMCL for prevalence of eye irritation; data from Kulle et al. (1987)

Dose	Est. prob.	Expected	Observed	Size	Residual
0	0.0277	0.526	1	19	0.663
0.5	0.0862	0.862	0	10	-0.971
1	0.2082	3.955	5	19	0.59
2	0.6143	11.672	10	19	-0.788
3	0.9183	8.265	9	9	0.895

Derivation of BMC and BMCL for PEFR in Children (Krzyzanowski et al., 1990)

A cross-sectional study of residential formaldehyde exposure in a large population-based sample observed a linear relationship between increased formaldehyde exposure and decreased peak expiratory flow rate (PEFR) among children exposed to average concentrations of 0.032 mg/m^3 (26 ppb) (Krzyzanowski et al., 1990). This study of effects in a residential population used a thorough exposure assessment protocol and repeated measurements of PEFR, thus, enhancing the ability to detect an association at the lower concentrations found in the homes. Declines in peak expiratory flow rate (PEFR) were associated with increases in 2-week average indoor residential formaldehyde concentrations, with greater declines observed in children (5–15 years of age, *n* = 208 in analytical data set) compared to adults (Krzyzanowski et al., 1990). Mean formaldehyde levels were 26 ppb (0.032 mg/m^3), and more than 84% of the homes had concentrations 40 ppb (0.049 mg/m^3) and lower.

EPA calculated the concentration at which a 10% decrement in pulmonary function would be expected. In this derivation, 10% decrement in a continuous response is considered to be the benchmark response (BMR). A $BMC_{10\%}$ and $BMCL_{10\%}$ were determined from the regression coefficient from a random effects model of PEFR among children reported by the study authors. Statistical models which adjusted for important covariates (including smoking status, SES, NO_2 levels, episodes of acute respiratory illness, and the time of day) did not identify any potential confounders and those covariates were not included in the final model.

$$y = 349.6 - 1.28 * (household\ formaldehyde) - 6.1 * (morning) + 0.09 * (bedroom\ formaldehyde) * (morning) + 0.0031 * (bedroom\ formaldehyde)^2 * (morning) + 4.59 * (morning) * (asthma) - 1.45 * (bedroom\ formaldehyde) * (morning) * (asthma) + 0.031\ (bedroom\ formaldehyde)^2 * (morning) * (asthma) \quad \text{(B-6)}$$

where *y* = PEFR (L/min); household formaldehyde = 2-week household mean concentration; morning = time of PEFR measurement (0,1); 2-week bedroom mean concentration; current asthma = doctor's diagnosis and current status (0,1).

For the purpose of deriving a point of departure for indoor formaldehyde, the primary estimate of the point of departure was computed for household formaldehyde with *morning* = 0 and *asthma* =0. The regression coefficient (β) for household formaldehyde was -1.28 ± 0.46 L/minute-ppb and the 95% one-sided upper bound on the regression coefficient was -2.04 L/minute-ppb;

$$\beta - (critical\ value\ for\ one - tailed\ \alpha\ of\ 0.05 * s.e.of\ \beta) = -1.28 - (1.645 * 0.46) = \mathbf{-2.04} \quad \text{(B-7)}$$

Based on the background PEFR of 349.6 L/minute, a 10% decrement is 35 L. Dividing 35 L by the regression coefficient for household formaldehyde of -1.28 L/minute-ppb (i.e., -1.28 L/(minute*ppb)), the change in formaldehyde concentration resulting in a 10% decrement in PEFR is 27 ppb which is equivalent to 0.033 mg/m^3. The BMCL resulting in a 10% decrease from a background of 349.6 L/minute is 17 ppb (35 L/minute divided by -2.04 L/minute-ppb), which is equivalent to 0.021 mg/m^3.

In order to estimate how much more sensitive asthmatic children were to formaldehyde, household and bedroom formaldehyde concentrations were assumed to be the same and *morning* = 1 and *asthma* = 1. Solving the final regression model for these realizations of *household formaldehyde*, *bedroom formaldehyde*, *morning,* and *asthma* yield the following:

$$\begin{aligned} -35\ \text{L/min} = {} & -1.28 * (household\ formaldehyde) - 6.1 * (1) + 0.09 \\ & * (household\ formaldehyde) * (1) + 0.0031 * (household\ formaldehyde)^2 * (1) \\ & + 4.59 * (1) * (1) - 1.45 * (household\ formaldehyde) * (1) * (1) \\ & + 0.031\ (household\ formaldehyde)^2 * (1) * (1) \end{aligned} \quad \text{(B-8)}$$

which simplifies to:

$$-35 \frac{\text{L}}{\text{min}} = 0.0341 * (household\ formaldehyde)^2 - 2.64 * (household\ formaldehyde) - 1.51 \quad \text{(B-9)}$$

Solving for *household formaldehyde* yields a $BMC_{10\%}$ (asthmatics) resulting in a 10% decrease from a background PEFR of 349.6 L/minute of 16 ppb given that asthmatic children were more sensitive to the respiratory effects of formaldehyde exposure than were children in general who had $BMC_{10\%}$ of 27 ppb.

Derivation of a BMC and BMCL for Asthma Exacerbation in Children with Asthma (Venn et al., 2003)

Venn et al. (2003) studied how indoor formaldehyde exposures affected the proportion of childhood asthma cases who reported symptoms of asthma attacks (asthma exacerbation). During an asthma attack, the muscles of the airways constrict thereby limiting air flow and the cells in the airway produce mucus which further restricts the passage of air. Symptoms included any of the following: wheezing, chest tightness, breathlessness, or cough (Venn et al., 2003). According to the Centers for Disease Control and Prevention (Moorman et al., 2012), more than 50% of children with asthma experienced at least one asthma attack in the previous 12 months yielding an annual rate of asthma attacks in the general population of children of more than 5%. Approximately 10% of children with asthma suffer an asthma attack resulting in a visit to the emergency room each year. The annual mortality rate from asthma among children is 2–3 per million (Moorman et al., 2012).

Venn et al. (2003, see Table B-8, see Table B-8) divided the children's bedroom formaldehyde exposures into quartiles and reported a statistically significant exposure-response trend of increasing risk of symptoms of an asthma attack with increasing quartiles of formaldehyde concentrations (p=0.03) and then fit a regression model to estimate the "per quartile" increase in risk. Venn et al. (2003) identified similar exposure-response functions for night-time and daytime symptoms of an asthma attack (asthma exacerbation) in children with asthma[28]: for night-time symptoms, the odds ratio (OR) per exposure quartile increase in formaldehyde concentration was 1.45 (95% CI: 1.06–1.98); for daytime symptoms, the OR per exposure quartile was 1.40 (95% CI: 1.00–1.94)[29]. Results were adjusted for age, sex, and socioeconomic status. Dampness was also reported to be a risk factor for symptoms of an asthma attack; however, further adjustment of the formaldehyde results for dampness made little difference (Venn et al., 2003). No effect of other volatile organic compounds or nitrogen dioxide on the risk of asthma attacks was found.

As the formaldehyde measures were taken in the children's bedrooms, the RfC derivation is based on the exposure-response function for night-time symptoms of an asthma attack. The following table summarizes the results from Venn et al. (2003) specific to the exposure-response relationship for night-time symptoms of asthma attacks in children with asthma. Note that, by definition, the OR reported for each exposure level is relative to the odds of being a case in the reference category, which is the lowest quartile of exposure. In Venn et al. (2003), the reference category is defined as exposures within the range 0-16 μg/m^3. The median concentration within this range was 12.24 μg/m^3 (Venn, 2012). In order to estimate the OR per unit increase in formaldehyde concentration from the reported effect per unit increase in quartile of formaldehyde

[28]Cases were defined as those whose doctors had prescribed asthma drug treatment at the time of the study (including the preceding year) (Venn et al., 2003).

[29]Exposure measurements, pulmonary function measurements, and symptoms of asthma attacks were measured over a 4-week period.

exposure, the difference in each quartile's median formaldehyde concentration was computed by subtracting 12.24 μg/m^3 from each quartile median.

Table B-8. Modeled effect estimates for night-time symptoms of an asthma attack; data from Venn et al. (2003)

Exposure quartile[a] (μg/m^3)	Quartile median[b] (μg/m^3)	Quartile median > reference quartile (μg/m^3)	OR by quartile[a]	Lower bound OR by quartile	Upper bound OR by quartile	Modeled OR[c]	Lower bound modeled OR[c]	Upper bound bodeled OR[c]
0–16	12.24	0	1			1		
16.1–22	19.23	6.99	1.4	0.54	3.62	1.45	1.06	1.98
22.1–32	26.55	14.31	1.61	0.62	4.19	2.10	1.12	3.92
32+	41.02	28.78	3.33	1.23	9.01	3.05	1.19	7.73

[a] Venn et al. (2003); [b] Venn (2012); [c] Venn et al. (2003) OR per increasing quartile = 1.45 (95% CI: 1.06–1.98).

EPA considered multiple methodologies for identifying a point of departure for this health endpoint. If the information provided by Venn et al. (2003) had been limited to just the quartile-specific results, then the one method might have used the results from Table B-8 of Venn et al. (2003) which show the first statistically significant effect occurring in the highest exposure group with a quartile mean of 41.02 μg/m^3 which could represent the LOAEL and thus the corresponding NOAEL could be the quartile mean of the third exposure group at 26.55 μg/m^3. However, because Venn et al. (2003) also reported a statistically significant exposure-response function (*p*-trend = 0.02) with OR=1.45 per exposure quartile (95% CI: 1.06–1.98), it is not reasonable to assume there is no effect at the median of the third quartile because the reported OR for this quartile was 1.61 (95% CI: 0.62 – 4.19) and the reported exposure-response function corresponds to a modeled OR=2.10 (95% CI: 1.12–3.92). Likewise, for the second quartile with a quartile-specific result of OR=1.4 (95% CI: 0.54–3.62), rather than evidence of "no effect," the reported exposure-response function indicates a modeled OR = 1.45 (95% CI: 1.06–1.98), which is consistent with the second quartile-specific results of OR = 1.4 but has narrower confidence intervals due to the use of data from all the quartiles rather than just a comparison of the second quartile to the first.

The reported exposure-response function from Venn et al. (2003) appears to be a more precise estimate of the exposure-response relationship for night-time symptoms of poor asthma control in children with asthma. In order to estimate a point of departure, the units of 'per quartile' need to be defined in terms of "per μg/m^3." As the magnitude of the increase in exposure from the median of the first quartile to the median of the second quartile is 6.99 μg/m^3, an estimate of the effect of exposure per μg/m^3 can be obtained by scaling the ln(OR) and its standard error by the difference in quartile medians. The OR = 1.45 per quartile (95% CI: 1.06–1.98) is first converted to the natural log scale as ln(OR) = 0.37156 per quartile (95%: 0.05827–0.68310), and then each term is multiplied by unity as expressed by [(1 quartile)/(6.99 μg/m^3)] to yield an effect of ln(OR) = 0.053156 (95% CI: 0.008336–0.09773), which when exponentiated back to the OR scale is

equivalent to an OR = 1.05 per μg/m^3 (95% CI: 1.01–1.10). This equivalent exposure-response function in terms of "per μg/m^3" retains the same *p*-trend value of 0.02 because the scaling cancels out.

According to Table B-8 in Venn et al. (2003), the prevalence of night-time asthma symptoms among the cases in the reference group is 0.41. Because the symptoms of an asthma attack among children with asthma is considered to be a frank effect (an overt of clinically apparent effect), a BMR of 5% was used to derive the POD for the derivation of the RfC (U.S. EPA, 2012). Using a BMR=5% extra risk for symptoms of an asthma attack, the prevalence of symptoms among the exposed at 5% extra risk compared to the prevalence of symptoms at zero exposure is:

$$\text{Extra Risk} = 0.05 = [P_{Exposed} - P_{Unexposed}] \div [1 - P_{Unexposed}] \text{ and } P_{Unexposed} = 0.41, \text{ then } P_{Exposed} = 0.4395. \quad \text{(B-10)}$$

$$\text{Find OR} = [P_{Exposed}/(1 - P_{Exposed})]/[P_{Unexposed}/(1 - P_{Unexposed})]$$
$$= [0.4395/(1 - 0.4395)]/[0.41/(1 - 0.41)] = 1.13 \quad \text{(B-11)}$$

For the derivation of the point of departure, here the benchmark concentration or BMC, note that the exposure-response function is defined relative to the reference group (those exposed to the first quartile of formaldehyde exposures) which experienced a median formaldehyde concentration of 12.24 μg/m^3 (Venn, 2012 personal communication personal communication). So in deriving the BMC, the first step is to estimate the magnitude of the concentration above the reference concentration of 12.24 μg/m^3, which corresponds to a 5% extra risk. For clarity, that value will be called the "interim BMC_{05}." The second step is to add that interim BMC_5 to the median formaldehyde concentration in the reference group. While it is possible that there are adverse effects of formaldehyde below the median formaldehyde concentration in the reference group, it should be understood that the methodology used in this derivation restricts the BMC to be greater than the median formaldehyde concentration in the reference group. The alternative would be to extrapolate the exposure-response function down from 12.24 μg/m^3 to either the background ambient formaldehyde concentration, or down to a concentration of zero.

To derive the interim BMC using the linear concentration-response function, solve for:

$$\text{OR corresponding to a 5\% extra risk} = 1.13 = (1.05 \text{ per } \mu g/m^3)*(\text{Interim } BMC_5)$$

$$\text{Interim } BMC_5 = 1.08 \ \mu g/m^3$$

To derive the interim BMCL using the linear concentration-response function, the one-sided 95% upper bound is needed (rather than the upper bound of the two-sided 95% CI around the OR). Using the one-sided 95% upper bound, which is 1.09 (calculation below)[30], solve for:

OR corresponding to a 5% extra risk = 1.13 = (1.09 per μg/m^3)*(Interim $BMCL_5$)

Interim $BMCL_5$ = 1.04 μg/m^3

Adding back the median formaldehyde concentration in the reference category (12.24 μg/m^3), the $BMCL_5$ value is 13.28 μg/m^3 and this value is selected as the point of departure for the cRfC.

B.1.3. Noncancer Estimates from Animal Toxicology Studies

Analysis of Respiratory Pathology Data from F344 and Wistar Rats

This appendix provides support to the decisions and details of modeling the respiratory pathology data in rats and mice in Section 2.1 for deriving candidate human inhalation RfCs based on these endpoints. These involve the following endpoints and studies: squamous metaplasia in F344 rats (Kerns et al., 1983), basal hyperplasia in Wistar rats (Woutersen et al., 1989), and squamous metaplasia in Wistar rats (Woutersen et al., 1989).

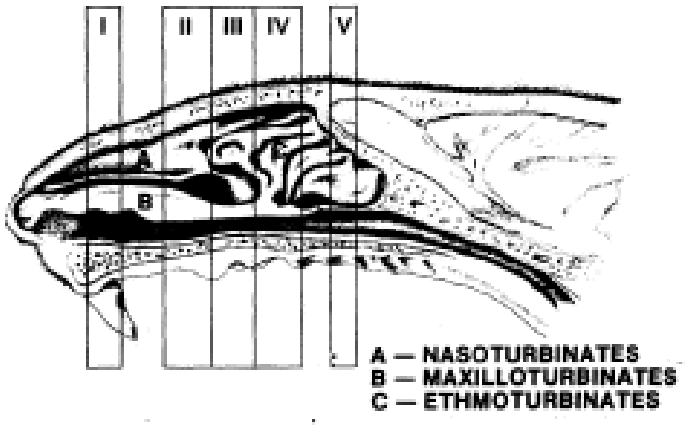


Figure B-6. Midsaggital section of rat nose showing section levels (Kerns et al., 1983) (nostril is to the left).

[30]To calculate the standard error of the ln(OR): [(ln(1.10)-ln(1.01)]/3.92=0.02178. Therefore, the 95% one-sided upper bound of the ln(OR) is [ln(OR)+1.645(0.02178)]=0.08461 and the 95% one-sided upper bound of the OR is 1.09.

Formaldehyde flux to the nasal lining was used in analyzing the dose-response data from Kerns et al. (1983) at the Level 1 cross section (Figure B-6) of the F344 rat nose, which is located in the front portion of the rat nose behind the nasal vestibule (Young, 1981). Kimbell et al. (2001b) modeled formaldehyde flux to the nasal lining; their flux estimates are shown in Figure B-7 as a contour plot of flux per ppm of exposure (note: only the lateral view of the three-dimensional surface is presented). These figures indicate that formaldehyde flux per ppm of exposure to the surface of the Level 1 section would correspond to the upper range (greater than approximately 1,750 pmol/mm^2-h-ppm) of flux estimates per ppm exposure. Kimbell et al. (2001b) divided their total flux (per ppm of exposure) range in the rat into 20 flux bins with the mean flux in bin 14 equal to 1,764 pmol/mm^2-h-ppm of exposure (see Table 1, see Table 1, Kimbell et al., 2001b). Therefore, we use flux estimates from flux bins 14-20 of their paper; the surface-area-weighted average flux per ppm of exposure in these flux intervals is 1,879.66 pmol/mm^2-h per ppm (i.e., 1,528.18 pmol/mm^2-h per mg/m^3) of exposure. Therefore, average flux in the Level 1 region corresponding to the $BMCL_{10}$ of 0.448 mg/m^3 is estimated to be 1,528.18 × 0.448–685 pmol/mm^2-hr.

In order to extrapolate the above BMCL to the human, one is interested in knowing the human exposure concentration at which some region in the human nose (see Figure B-7) is exposed to a formaldehyde flux of 685 pmol/mm^2-hr. This is estimated from Table 3 in Kimbell et al. (2001b), which tabulates formaldehyde flux to the human nasal lining at various inspiratory rates. At any given exposure, the anterior regions of the nose are subject to the highest concentrations of formaldehyde; therefore, we averaged the data from flux bins 17–20 in their tabulation, which receive the highest levels of flux. The average flux per ppm of exposure concentration in bins 17–20 in the human is 1,741 pmol/mm^2-h per ppm of exposure. Thus, the exposure concentration at which these regions would receive a flux of 685 pmol/mm^2-hr is 0.484 mg/m^3. This is the human BMCL corresponding to 0.10 extra risk, which was selected because the observed squamous metaplasia was determined to be of minimal-to-mild adversity.

As discussed in section 1.2.4 of the Toxicological Review, squamous metaplasia occurred in several sagittal cross sections (Level 1–5, depicted in Figure B-6) of the F344 rat nose in the Kerns et al. (1983) study. However, accurate estimates of formaldehyde flux over the nasal lining other than Level 1 were not available to EPA, and flux estimates provided in Kimbell et al. (,2001,054906} cannot be reliably used for the other cross-sections because of a lack of correspondence with the nasal regions in their paper. Therefore, only the squamous metaplasia data reported for Level 1 was carried forward in calculating a candidate RfC. Details of benchmark dose modeling for data on squamous metaplasia in F344 rat and squamous metaplasia and basal hyperplasia in Wistar rat are shown in Table B-9 and Figures B-8 to B-12.

Table B-9. Benchmark dose modeling of rat respiratory histopathological effects

Model	BMR	AIC	BMD	BMDL	Model fit	Best model	Notes
Squamous metaplasia in F344 rat (Level 1)							
Mstage k=2	0.10	97.779	0.351	0.281	Fig. 3		
Log-logistic	0.10	97.322	0.492	0.119	Fig. 3		BMD/BMDL > 4
Log-Probit	0.10	95.619	0.576	0.448	Fig. 4	√	Lowest AIC
Basal hyperplasia in Wistar rat (anterior, Levels 1 & 2)							
Mstage k=2	0.10	65.842	1.767	1.109			
Mstage k=1	0.10	63.846	1.676	1.108	Fig. 7	√	Lowest AIC
Log-logistic	0.10	65.975	1.633	0.711			
Squamous metaplasia in Wistar rat (anterior, Levels 1 & 2)							
Log-logistic	0.10	71.810	1.003	0.526	Fig. 8	√	Lowest AIC
Mstage k=2	0.10	72.157	0.917	0.376	Fig. 8		

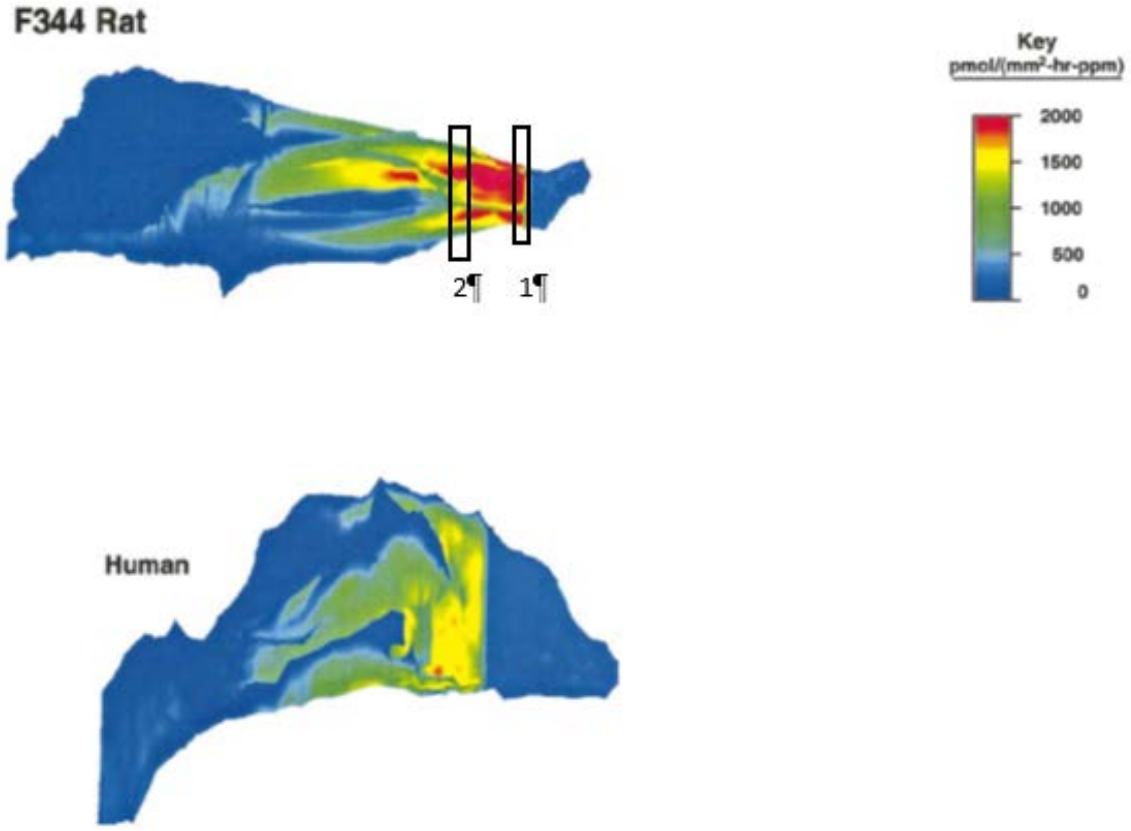


Figure B-7. Lateral view of contour plot of formaldehyde flux to the rat (on the top) and human nasal lining (on the bottom) using CFD modeling (Kimbell et al., 2001b) (nostril is to the right). The actual surface is three-dimensional. Flux at a site is linear with exposure concentration and is shown here in terms of per ppm; therefore, values shown here need to be multiplied by exposure concentration. Rectangular boxes on the rat mesh roughly estimate location of section Levels 1 & 2 in Kerns et al. (1983) (corresponding to Figure B-6).

1

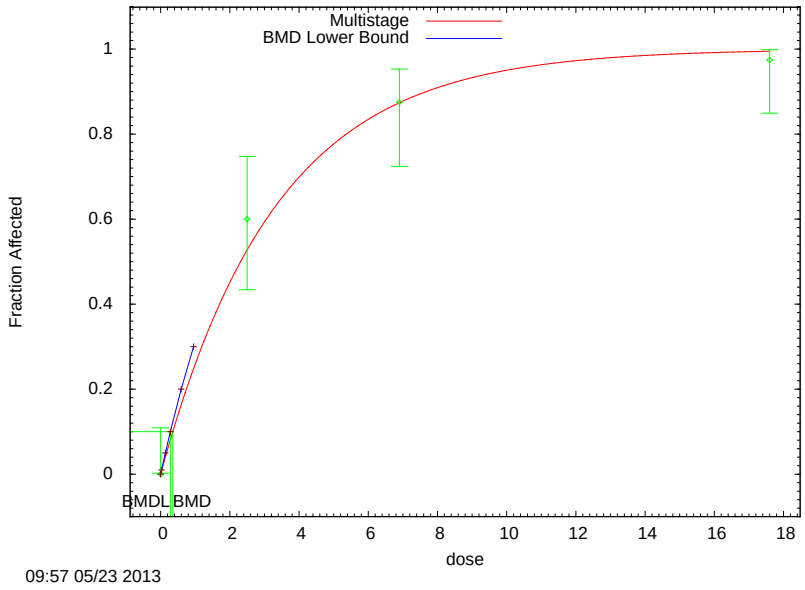


Figure B-8. Multistage model fit for Level 1 squamous metaplasia.

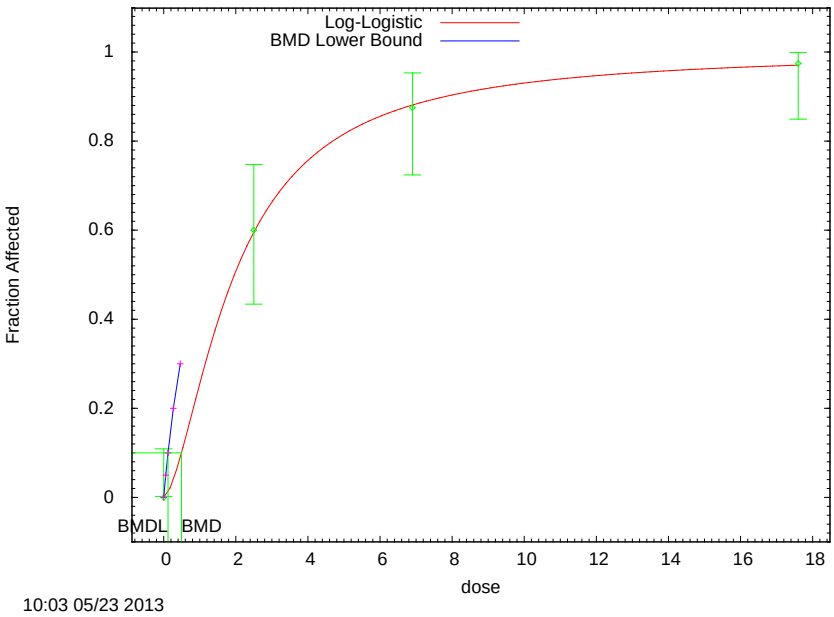


Figure B-9. Log-logistic (bottom panel) model fit for Level 1 squamous metaplasia.

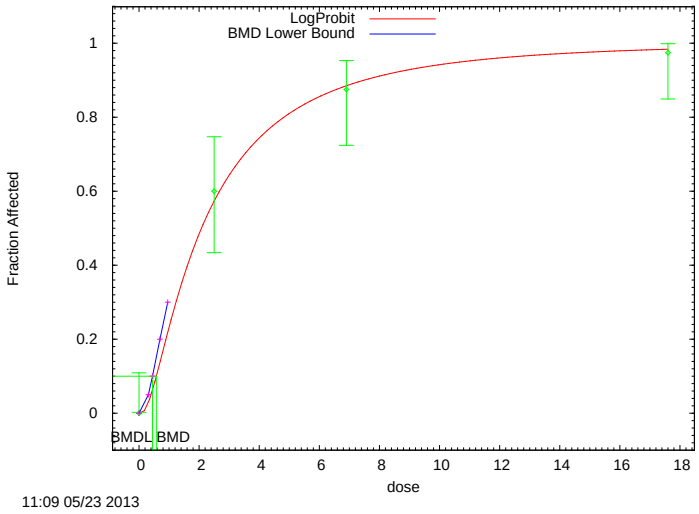


Figure B-10. Log-probit model fit for Level 1 squamous metaplasia.

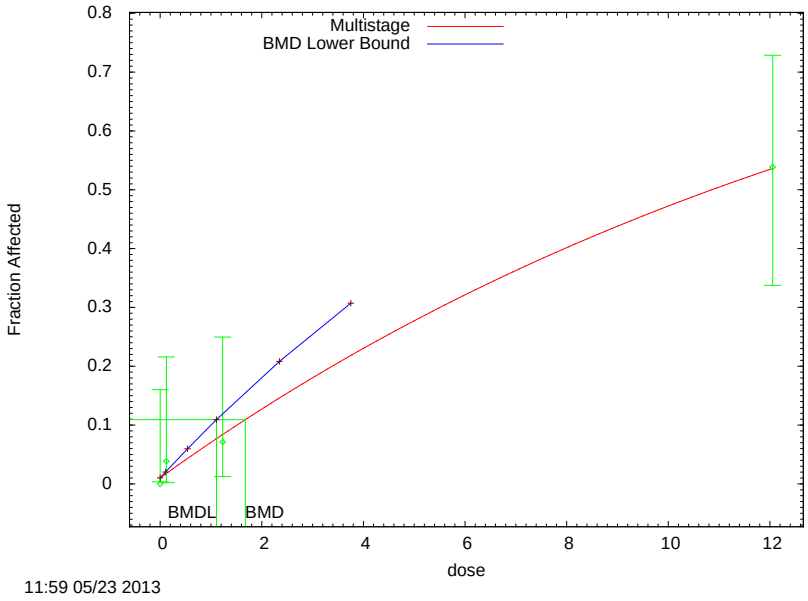


Figure B-11. Basal hyperplasia in Wistar rat (Woutersen et al., 1989): multistage model (*k*=1) fit.

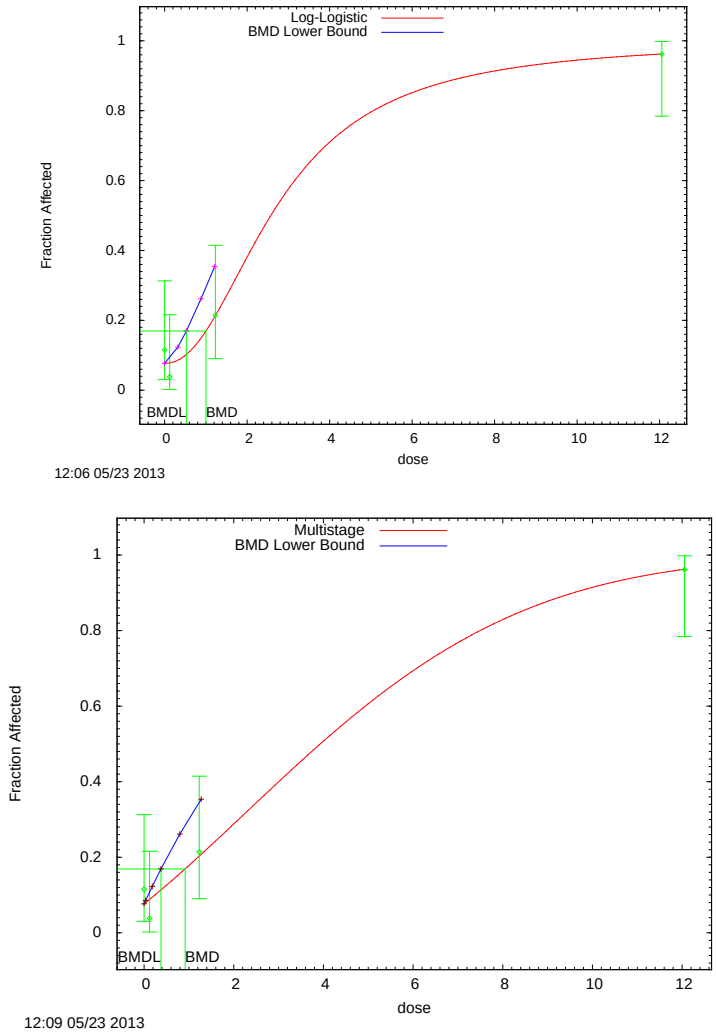


Figure B-12. Squamous metaplasia in Wistar rat (Woutersen et al., 1989): log-logistic (top panel) and multistage (bottom panel) model fit

Reproductive Toxicity in Males

Two studies reporting effects on the male reproductive system in rats were considered to be of sufficient quality for candidate reference value derivation (Ozen et al., 2005; Ozen et al., 2002). For each endpoint, the BMDL estimate (95% lower confidence limit on the BMD, as estimated by the profile-likelihood method) and AIC value were used to select a best-fit model from among the models exhibiting adequate fit. If the BMDL estimates were "sufficiently close," that is, differed by at most xx-fold, the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, the lowest BMDL was selected as the POD.

Table B-10. Endpoints selected for dose-response modeling for reproductive and developmental toxicity in animals

Species (strain)/Sex Endpoint	Concentrations and Effect Data			
Ozen et al. (2005), *Table 1*				
Rat (Wistar)/adult males, 13-wk exposure	Concentration (mg/m^3)[a]	0	1.462	2.924
Serum testosterone (ng/L)	No. of animals Mean ± SD	6 406.5 ± 41.20	6 244.0 ± 58.44	6 141.3 ± 20.97
Ozen et al. (2002), *Table 2*				
Rat (Wistar)/adult males, 13-wk exposure	Concentration (mg/m^3)[b]	0	2.905	5.810
Testis weight as percent of body weight	No. of animals Mean ± SD	7 0.91 ± 0.01	7 0.84 ± 0.03	7 0.82± 0.03
Ozen et al. (2002), *Table 2*				
Rat (Wistar)/adult males, 4-week exposure	Concentration (mg/m^3)[a]	0	2.905	5.810
Testis weight as percent of body weight	No. of animals Mean ± SD	7 0.94 ± 0.03	7 0.92 ± 0.02	7 0.91± 0.01

[a] Reported as 0, 5, and 10 ppm. Conversion: ppm*(30.02598/24.45)*(8 hrs/24 hrs)*(5 d/7d)
[b] Reported as 0, 12.2, and 24.4 mg/m^3. Conversion: (mg/m^3)*(8 hrs/24 hrs)*(5 d/7d)

Modeling Results

Below are tables summarizing the modeling results for the noncancer endpoints modeled. The following parameter restrictions were applied, unless otherwise noted:

- Dichotomous models: For the log-logistic and dichotomous Hill models, restrict slope ≥ 1; for the gamma and Weibull models, restrict power ≥ 1; for the multistage models, restrict betas ≥ 0.

- Continuous models: For the polynomial models, restrict the coefficients b1 and higher to be nonnegative or nonpositive if the direction of the adverse effect is upward or downward, respectively; for the Hill, power and exponential models restrict power ≥ 1.

Serum testosterone (Ozen et al., 2005)

For the BMD modeling of serum testosterone in male Wistar rats exposed to formaldehyde by inhalation for 13 weeks (Ozen et al., 2005), model fit to the mean responses was good. Fit of the models for variance was marginal because the reported sample estimates of standard deviations (SD) did not change monotonically with concentrations. Nevertheless, it is reasonable to accept the best fitting model because the estimated SD of 41.7 is closer to that reported for the control (41.2), meaning that the 1-SD BMR is estimated reasonably well. As both the means and the control SD are well estimated, the BMD is also estimated reasonably well.

Table B-11. Summary of BMD modeling results for serum testosterone in male Wistar rats exposed to formaldehyde by inhalation for 13 weeks (Ozen et al., 2005); BMR = 1 SD change from the control mean

Model[a]	Goodness of fit		BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2)[a]	**0.84**	**156.2**	**0.284**	**0.208**	Exponential Models 2 and 4 provided the best fit with identical AIC to 4 decimals (156.1811). Fit of Variance Models (Test 3) was marginal at p = 0.065 with constant variance and did not improve when variance was modeled as a power of means (P=0.050).
Exponential (M3)	NA[c]	158.1	0.314	0.209	
Exponential (M4)[b]	0.84	156.2	0.284	0.189	
Exponential (M5)[c]	NA				
Hill[c]	NA				
Polynomial 1°[d] Polynomial 2° Power	0.14	158.3	0.460	0.348	

[a]Constant variance models are presented (BMDS Test 3 p-value = 0.065), with the selected model in bold. Scaled residuals for selected model for concentrations 0, 1.462, and 2.924 mg/m^3 were –0.046, 0.15, and –0.13, respectively.

[b]For exponential model M4, the estimate of d, 1.0498, was close to a boundary (1) and parameter estimates were close to those for M2. The lower BMDL is a result of having one more free parameter (d) than M2.

[c] These models could not be fitted (more parameters than dose groups).

[d]For the power model, the power parameter estimate was 1 (boundary of parameter space). For the Polynomial 2 model, the b2 coefficient estimate was 0 (boundary of parameter space). Consequently, the models in this row reduced to the Polynomial 1° model.

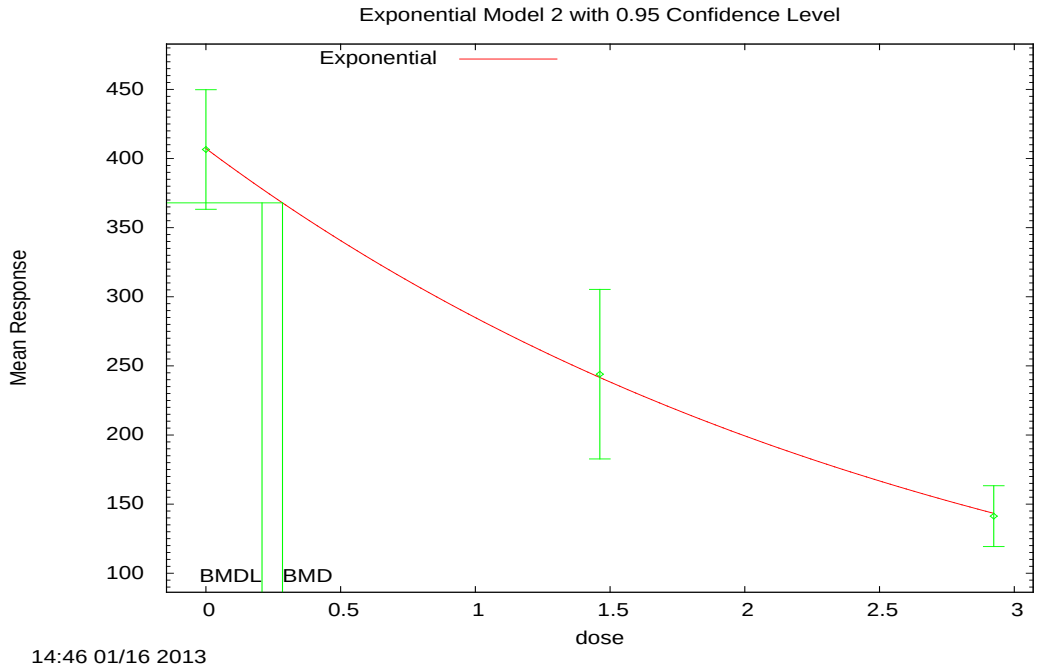


Figure B-13. Plot of mean response (serum testosterone, serum testosterone, Ozen et al., 2005) by concentration, with the fitted curve for Exponential Model 2 with constant variance. BMR = 1 SD change from the control mean. Concentrations are in mg/m^3.

Relative Testis Weight at 4 weeks (Ozen et al., 2002)

Models were fitted successfully to data for the 4-week exposure duration. Fit of the models for variance was marginal (*P*=0.026 with constant variance, *P*=0.047 with modeled variance). It may be reasonable to accept the best fitting model, because the estimated SDs and means are fairly close to the observed values. The customary BMR for body and organ weights is "10% relative deviation," (i.e., a 10% difference from the control mean). However, the change in means across the experimental doses was much less than 10% so the BMDs for 10% relative deviation (16–17 mg/kg-g) fall well above the highest dose (5.8 mg/kg-g), leading to unacceptable extrapolation. The table below reports only the BMDs for the 1-SD BMR.

Table B-12. Summary of BMD modeling results for relative testis weight in male Wistar rats exposed to formaldehyde by inhalation for 4 weeks (Ozen et al., 2002); BMR = 1-SD change from the control mean

Model[a]	Goodness of fit		BMD1SD (mg/kg-d)	BMDL1SD (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2)a	NA	-138.2	3.81	2.60	The Polynomial 1° model fits the means adequately, but the fit of the variance model is marginal at *P*=0.047.
Exponential (M3)	NA	-126.4	1,944	1.87	
Exponential (M4)[b]	NA	-126.4	NA	NA	
Exponential (M5)[c]	NAc	NA	NA	NA	
Hill[c]	NA	NA	NA	NA	
Polynomial 1[d] Polynomial 2°	**0.529**	**-138.2**	**3.841**	**2.636**	
Power[d]	<0.0001	-140.2	3.841	2.636	

[a] Variances were modeled as a power of the means (BMDS Test 3 *p*-value = 0.047), with the selected model in bold. Note that the power coefficient in the variance model was 18, which is a boundary artificially imposed by BMDS. Scaled residuals for selected model for concentrations 0, 2.905, and 5.81 mg/m^3.

[b]For exponential model M4, the estimate of *d*, 1.0498, was close to a boundary (1) and parameter estimates were close to those for M2. The lower BMDL is a result of having one more free parameter (*d*) than M2.

[c] These models could not be fitted (more parameters than dose groups).

[d]For the power model, the power parameter estimate was 1 (boundary of parameter space). For the Polynomial 2 model, the b2 coefficient estimate was 0 (boundary of parameter space). Consequently, the models in this row reduced to the Polynomial 1° model.

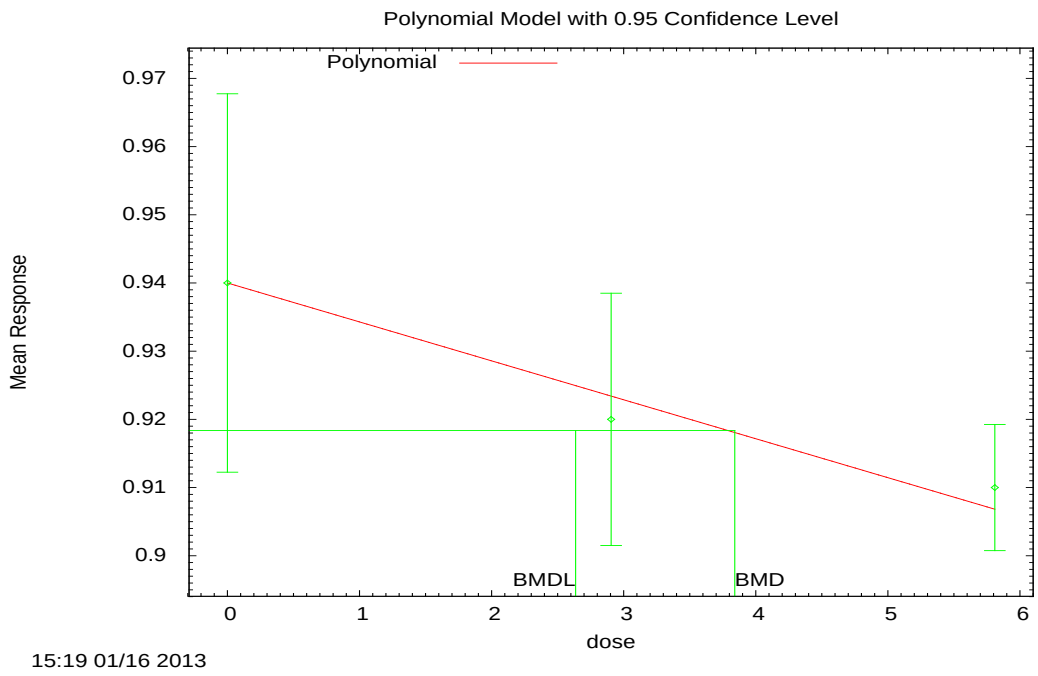


Figure B-14. Plot of mean response (relative testis weight, relative testis weight, Ozen et al., 2002) by concentration, with the fitted curve for a linear model with modeled variance. BMR = 1 SD change from the control mean. Concentrations are in mg/m^3.

Relative Testis Weight at 13 weeks (Ozen et al., 2002)

Most BMDS models could not be fitted successfully to data for testis weight as a percentage of body weight (Ozen et al., 2002) at the 13-week exposure duration because they reduce to linear models that had large scaled residuals (poor fit). The Exponential Model 4 did achieve an acceptable fit, but the likelihood ratio goodness-of-fit test had zero degrees of freedom. Therefore, Exponential Model 4 was selected. The target BMR, 10% relative change from the control mean, fell outside the range of observed responses: the control mean was 0.91 and the response at the high concentration was 0.84 (8% below the control mean). The BMD was 9.99 while the highest concentration was 5.81.

An alternative POD is the LOAEL. EPA calculations indicate that if the data are normally distributed (unverified, but plausible for relative weights), the response at the first concentration represents a decrease of 7.7% below control (95% confidence interval 4.6% to 11%), and the response at the second concentration represents a decrease of 11% (95% confidence interval 7.9% to 14%). The response at the second concentration is closest to the target BMR for organ weights (10% decrease), so the second concentration (5.81 mg/m^3) would be used as the biologically relevant POD.

Table B-13. Model predictions for relative testis weight (Ozen et al., 2002)

Model[a]	Goodness of Fit		BMD_{1SD} (mg/m^3)	$BMDL_{1SD}$ (mg/m^3)	BMD_{10RD} (mg/m^3)	$BMDL_{10RD}$ (mg/m^3)	Basis for Model Selection
	p-value	AIC					
Exponential (M2) Exponential (M3)[b]	0.011	-129.70	0.574	0.326	4.68	3.74	Smallest AIC
Exponential (M4)	**N/A[c]**	**-134.46**	**0.204**	**5.02×10^{-04}[d]**	**9.99**	**3.24**	
Power	0.00705	-128.90	0.621	0.348	4.70	3.75	
Polynomial 2[e] Linear	0.00598	-128.90	0.621	0.348	4.70	3.75	

[a]Modeled variance case presented (BMDS Test 2 *p*-value = 0.0183), selected model in bold; scaled residuals for selected model for concentrations 0, 2.905, and 5.81 mg/m^3 were -0.01397, 0.2209, and -0.2285, respectively.
[b]For the Exponential (M3) model, the estimate of *d* was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]Model curvature becomes extreme near the origin, resulting in a very small BMDL for the 1-SD BMR. Model 4 is the only one with curvature; the other models are linear and do not fit as well.
[e]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

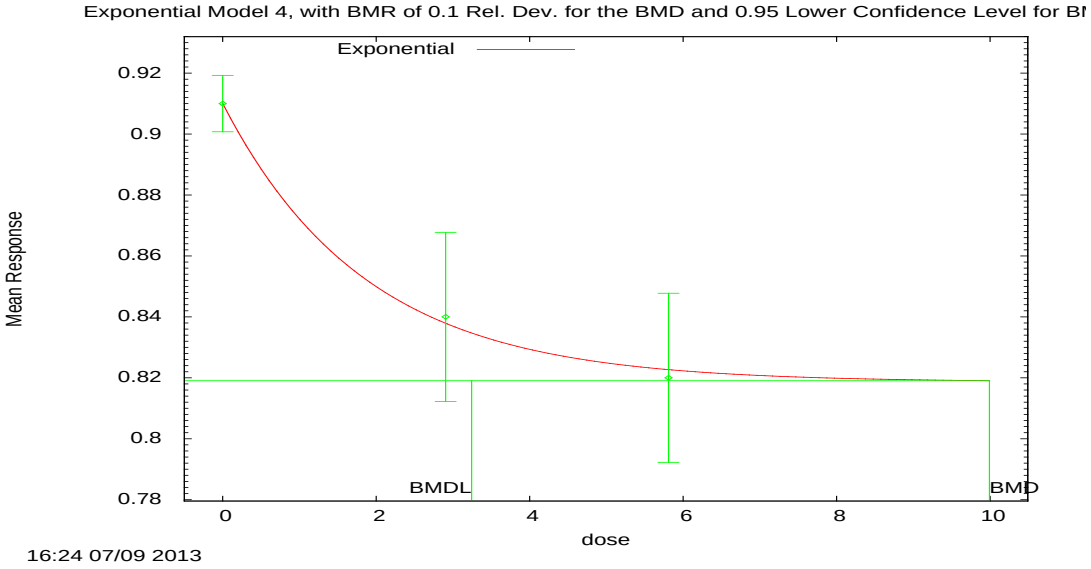


Figure B-15. Plot of mean response by concentration, with fitted curve for selected model; concentration shown in mg/m^3.

BMDS Modeling Output

Exponential Model. (Version: 1.9; Date: 01/29/2013)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
Parameter *d* is defined *d*=1; it is, therefore, not estimated (it is estimated for M5).
A modeled variance is fit.

Benchmark Dose Computation.
BMR = 10% relative deviation
BMD = 9.99109
BMDL at the 95% confidence level = 3.24373

Table B-14. Parameter estimates

Variable	Estimate	Default initial parameter values
lnalpha	-11.5414	-11.2791
rho	-23.5629	-22.6938
a	0.91005	0.9555
b	0.535554	0.280827
c	0.899523	0.817323
d	1	1

Table B-15. Table of data and estimated values of interest

Dose	N	Obs mean	Est mean	Obs std dev	Est std dev	Scaled resid
0	7	0.91	0.91	0.01	0.009464	-0.01397
2.905	7	0.84	0.8379	0.03	0.02504	0.2209
5.81	7	0.82	0.8227	0.03	0.03108	-0.2285

Table B-16. Likelihoods of interest

Model	Log(likelihood)	# Params	AIC
A1	68.44598	4	-128.892
A2	72.44658	6	-132.8932
A3	72.0827	5	-134.1654
R	54.58803	2	-105.1761
4	72.22982	5	-134.4596

Table B-17. Tests of interest

Test	-2 Log(likelihood ratio)	Test df	p-value
Test 1	**35.72**	**4**	**<0.0001**
Test 2	8.001	2	0.0183
Test 3	0.7278	1	0.3936
Test 6a	-0.2942	0	N/A

B.2. DOSE-RESPONSE ANALYSIS FOR CANCER

B.2.1. Cancer Estimates from Observational Epidemiology Studies

Illustration of Life-table Analysis for NPC Risk in Humans Based on Data in Beane Freeman, (2013)

A spreadsheet illustrating the calculation for the derivation of the lower 95% bound on the effective concentration associated with a 0.05% extra risk (LEC_{0005}) for nasopharyngeal carcinoma (NPC) incidence is presented in Table B-18.

Table B-18. Extra risk calculation[a] for environmental exposure to 0.0550 ppm formaldehyde (the LEC_{0005} for NPC incidence)[b] using a log-linear exposure-response model based on the cumulative exposure trend results of Beane Freeman (2013), as described in Section 2.2.1

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Interval number (i)	Age interval	All-cause mortality (×10^5/yr)	NPC incidence (×10^5/yr)	All cause hazard rate (h*)	Prob of surviving interval (q)	Prob of surviving up to interval (S)	NPC cancer hazard rate (h)	Cond prob of NPC incidence in interval (Ro)	Exp duration mid interval (xtime)	Cum exp mid interval (xdose)	Exposed NPC hazard rate (hx)	Exposed all cause hazard rate (h*x)	Exposed prob of surviving interval (qx)	Exposed prob of surviving up to interval (Sx)	Exposed cond prob of NPC in interval (Rx)
1	<1	623.4	0.02	0.0062	0.9938	1.0000	0.00000	0.000000	0	0.0000	0.0000	0.0062	0.9938	1.0000	0.00000
2	1-4	26.5	0.05	0.0011	0.9989	0.9938	0.00000	0.000002	0	0.0000	0.0000	0.0011	0.9989	0.9938	0.00000
3	5-9	11.5	0.06	0.0006	0.9994	0.9927	0.00000	0.000003	0	0.0000	0.0000	0.0006	0.9994	0.9927	0.00000
4	10-14	14.3	0.11	0.0007	0.9993	0.9922	0.00001	0.000005	0	0.0000	0.0000	0.0007	0.9993	0.9922	0.00001
5	15-19	49.4	0.15	0.0025	0.9975	0.9915	0.00001	0.000007	2.5	0.4182	0.0000	0.0025	0.9975	0.9915	0.00001
6	20-24	86.5	0.17	0.0043	0.9957	0.9890	0.00001	0.000008	7.5	1.2547	0.0000	0.0043	0.9957	0.9890	0.00001
7	25-29	96.0	0.18	0.0048	0.9952	0.9847	0.00001	0.000009	12.5	2.0911	0.0000	0.0048	0.9952	0.9847	0.00001
8	30-34	110.2	0.30	0.0055	0.9945	0.9800	0.00002	0.000015	17.5	2.9276	0.0000	0.0055	0.9945	0.9800	0.00002
9	35-39	138.8	0.54	0.0069	0.9931	0.9746	0.00003	0.000026	22.5	3.7641	0.0000	0.0069	0.9931	0.9746	0.00003
10	40-44	201.1	0.80	0.0101	0.9900	0.9679	0.00004	0.000039	27.5	4.6005	0.0001	0.0101	0.9900	0.9679	0.00005
11	45-49	324.0	1.07	0.0162	0.9839	0.9582	0.00005	0.000051	32.5	5.4370	0.0001	0.0162	0.9839	0.9582	0.00008
12	50-54	491.7	1.48	0.0246	0.9757	0.9428	0.00007	0.000069	37.5	6.2734	0.0001	0.0246	0.9757	0.9428	0.00011
13	55-59	711.7	1.70	0.0356	0.9650	0.9199	0.00009	0.000077	42.5	7.1099	0.0001	0.0356	0.9650	0.9198	0.00013
14	60-64	1,015.8	1.85	0.0508	0.9505	0.8878	0.00009	0.000080	47.5	7.9464	0.0002	0.0509	0.9504	0.8876	0.00014
15	65-69	1,527.6	2.19	0.0764	0.9265	0.8438	0.00011	0.000089	52.5	8.7828	0.0002	0.0765	0.9264	0.8436	0.00017
16	70-74	2,340.9	2.08	0.1170	0.8895	0.7817	0.00010	0.000077	57.5	9.6193	0.0002	0.1172	0.8894	0.7815	0.00016
17	75-59	3,735.4	1.85	0.1868	0.8296	0.6954	0.00009	0.000059	62.5	10.4557	0.0002	0.1869	0.8295	0.6951	0.00013
18	80-84	6,134.1	1.86	0.3067	0.7359	0.5769	0.00009	0.000046	67.5	11.2922	0.0002	0.3068	0.7358	0.5766	0.00011
							Ro =	0.000662						**Rx =**	0.001163
Extra Risk = (Rx−Ro)/(1−Ro) = 0.0005															

Column A: Interval index number (i).

Column B: 5-year age interval (except <1 and 1−4) up to age 85.

Column C: All-cause mortality rate for interval i (× 105/year) (2010 data from NCHS).

Column D: NPC incidence rate for interval i (× 105/year) (2000-2010 SEER data).

Column E: All-cause hazard rate for interval i (h*i) (= all-cause mortality rate × number of years in age interval).[c]

Column F: Probability of surviving interval i without being diagnosed with NPC (qi) (= exp(−h*i)).

Column G: Probability of surviving up to interval i without having been diagnosed with NPC (Si) (S1 = 1; Si = Si−1 × qi−1, for i>1).

Column H: NPC incidence hazard rate for interval i (hi) (= NPC incidence rate × number of years in interval).

Column I: Conditional probability of being diagnosed with NPC in interval i (= (hi/h*i) × Si × (1−qi)), i.e., conditional upon surviving up to interval i without having been diagnosed with NPC [Ro, the background lifetime probability of being diagnosed with NPC, is the sum of the conditional probabilities across the intervals].

Column J: Exposure duration (in years) at mid-interval (xtime).

Column K: Cumulative exposure mid-interval (xdose) (= exposure level (i.e., 0.0550 ppm) × 365/240 × 20/10 × xtime) [365/240 × 20/10 converts continuous environmental exposures to corresponding occupational exposures].

Column L: NPC incidence hazard rate in exposed people for interval i (hxi) (= hi × (1 + β × xdose), where β = 0.04311 + (1.645 × 0.01865) = 0.07379 per ppm × year) [0.04311 per ppm × year is the regression coefficient obtained, along with its SE of 0.01865, from Dr. Beane Freeman (see Section 2.2.1). To estimate the LEC_{0005} (i.e., the 95% lower bound on the continuous exposure giving an extra risk of 0.05%), the 95% upper bound on the regression coefficient is used (i.e., MLE + 1.645 × SE)].

Column M: All-cause hazard rate in exposed people for interval i (h*xi) (= h*i + (hxi − hi)).

Column N: Probability of surviving interval i without being diagnosed with NPC for exposed people (qxi) (= exp(−h*xi)).

Column O: Probability of surviving up to interval i without having been diagnosed with NPC for exposed people (Sxi) (Sx1 = 1; Sxi = Sxi−1 × qxi-1, for i>1).

Column P: Conditional probability of being diagnosed with NPC in interval i for exposed people (= (hxi/h*xi) × Sxi × (1−qxi)) [Rx, the lifetime probability of being diagnosed with NPC for exposed people = the sum of the conditional probabilities across the intervals].

[a]Using the methodology of BEIR IV (,1988, 199516).

[b]The estimated 95% lower bound on the continuous exposure level of formaldehyde that gives a 0.05% extra lifetime risk of NPC.

[c]For the cancer incidence calculation, the all-cause hazard rate for interval i should technically be the rate of either dying of any cause or being diagnosed with the specific cancer during the interval [i.e., (the all-cause mortality rate for the interval + the cancer-specific incidence rate for the interval − the cancer-specific mortality rate for the interval [so that a cancer case isn't counted twice, i.e., upon diagnosis and upon death]) × number of years in interval]. This adjustment was ignored here because the NPC incidence rates are small compared to the all-cause mortality rates.

MLE = maximum likelihood estimate; SE = standard error

B.2.2. Cancer Estimates from Animal Toxicology Studies Using Biologically Based Dose Response (BBDR) Modeling

Biologically based dose-response models were developed in a series of papers and in a health assessment report by scientists at the Chemical Industry Institutes of Toxicology (CIIT) (Conolly et al., 2004, 2003; Conolly, 2002; Kimbell et al., 2001b; Kimbell and Subramaniam, 2001; Overton et al., 2001; Conolly et al., 2000; CIIT, 1999) to interpret the tumor incidence observed in F344 rats in two long-term bioassays (Monticello et al., 1996; Kerns et al., 1983) and extrapolate risk from rats to humans. The CIIT modeling and available data, and alternatives based on their original model were evaluated extensively for the purpose of this assessment and used in calculating the cancer potency. This section of the appendix separately addresses the BBDR models developed for the F344 rat and the human, and in each case: first provides clarifying details regarding the model, then summarizes all the issues evaluated, and finally provides detailed evaluations of key issues.

Model Structure and Calibration in Conolly et al. (2004, 2003)

In Conolly et al. (2003), tumor incidence data in the above long-term bioassays were modeled by using an approximation of the two-stage clonal growth model (Moolgavkar et al., 1988) and allowing formaldehyde to have directly mutagenic action. Conolly et al. (2003) combined these data with historical control data on 7,684 animals obtained from National Toxicology Program (NTP) bioassays. These models are based on the Moolgavkar, Venzon, and Knudson (MVK) stochastic two-stage model of cancer (Moolgavkar et al., 1988; Moolgavkar and Knudson, 1981; Moolgavkar and Venzon, 1979), which accounts for growth of a pool of normal cells, mutation of normal cells to initiated cells, clonal expansion and death of initiated cells, and mutation of initiated cells to fully malignant cells. The following notations are used in the rest of this appendix:

- N cell, normal cell
- I cell, initiated cell
- LI, labeling index (number of labeled cells/(number labeled + unlabeled cells))
- ULLI, unit length labeling index (number labeled cells/length of basement membrane)
- N, number of normal cells that are eligible for progression to malignancy
- α_N, division rate of normal cells (hours^{-1})
- μ_N, rate at which an initiated cell is formed by mutation of a normal cell (per cell division of normal cells)
- α_I, division rate of an initiated cell (hours^{-1})
- β_I, death rate of an initiated cell (hours^{-1})
- μ_I, rate at which a malignant cell is formed by mutation of an initiated cell (per cell division of initiated cells)

Cell replication rates and DPX concentrations are driven by local dose, which is formaldehyde flux to each region of nasal tissue expressed as pmol/mm^2-hour and predicted by computational fluid dynamics (CFD) modeling using anatomically accurate representations of the

nasal passages (see Appendix A.2.12). In the CIIT model, cell division and mutation is treated as a function of local flux. The spatial distribution of formaldehyde over the nasal lining was characterized by partitioning the nasal surface by formaldehyde flux to the tissue (rate of gas absorbed per unit surface area of the nasal lining), resulting in 20 "flux bins" with low bin numbers associated with low flux values. Each bin is comprised of elements of the nasal surface, which are not necessarily contiguous, that receive a particular interval of formaldehyde flux per ppm of exposure concentration (Kimbell et al., 2001b). Because formaldehyde mass transfer is airflow-limited, flux is assumed to scale linearly with inhaled exposure concentration (ppm); accordingly it is expressed in the CFD modeling in (Kimbell et al., 2001b) in terms of pmol/mm^2-hr-ppm, and the spatial coordinates of elements comprising a particular flux bin are fixed for all exposure concentrations. Because there is a decreasing gradient of flux from proximal to distal regions of the nose, the nasal surface area attributed to a bin drops sharply with increasing flux bin numbers (see Fig. 4 in (Kimbell et al., 2001b)).

Inputs to the model: The inputs to the two-stage cancer modeling consisted of results from other model predictions as well as empirical data. These included: regional uptake of formaldehyde in the respiratory tract predicted by using CFD modeling in the F344 rat and human (Kimbell et al., 2001b; Kimbell and Subramaniam, 2001; Overton et al., 2001; Subramaniam et al., 1998) discussed in Appendix A.2; concentrations of DPXs predicted by a PBPK model (Conolly et al., 2000) calibrated to fit the DPX data in F344 rat and rhesus monkey (Casanova et al., 1994; Casanova et al., 1991) and subsequently scaled up to humans; and cell division rates for normal cells (α_N) inferred from labeling index data on rats exposed to formaldehyde (Monticello et al., 1996; Monticello et al., 1991; Monticello et al., 1990).

Calibration: The rat model in Conolly et al. (2003) involved six unknown statistical parameters that were estimated by fitting the model to the rat formaldehyde bioassay data shown in Table 2-20 of the main document (Monticello et al., 1996; Kerns et al., 1983) plus historical data from several thousand control animals from all the rat bioassays conducted by the NTP. These NTP bioassays were conducted from 1976 through 1999 and included 7,684 animals with an incidence of 13 SCCs (i.e., 0.17% incidence). The resulting model predicts the probability of a nasal SCC in the F344 rat as a function of age and exposure to formaldehyde. The fit of the Conolly et al. (2003) model to the tumor incidence data is shown in Figure 2-4 of the main document.

Modeling formaldehyde's mutational action: Formaldehyde interacts with DNA to form DPXs. In Conolly et al. (2003), DPX formation is considered proportional to the intracellular dose of formaldehyde related to its directly mutagenic action. Casanova et al. (1994; 1989) carried out two studies of DPX measurements in F344 rats. In the first study, rats were exposed to concentrations of 0.3, 0.7, 2, 6, and 10 ppm for 6 hours and DPX measurements were made over the whole respiratory mucosa of the rat, while in the second study, the exposure was to 0.7, 2, 6, or 15 ppm formaldehyde for 3 hours and measurements were made at "high" and "low" tumor sites. Conolly et al. (2000) used data from the second study to develop a PBPK model that predicted the time course

of DPX concentrations as a function of regional formaldehyde flux (estimated in the CFD modeling and expressed as pmol/mm^2-hour). In the two-stage clonal expansion model the mutation rate of normal and initiated cells were defined as the same linear function of DPX concentration as follows:

$$\mu_N = \mu_I = \mu_{Nbasal} + KMU \times DPX \quad \text{(B-12)}$$

The unknown constants μ_{Nbasal} and KMU were estimated by fitting model predictions to the tumor bioassay data.

Use of labeling data: Cell replication rates in Conolly et al. (2003) were obtained by pooling labeling data from two phases of a labeling study in which male F344 rats were exposed to formaldehyde gas at similar concentrations (0, 0.7, 2.0, 6.0, 10.0, or 15.0 ppm). The first phase employed injection labeling with a 2-hour pulse labeling time, and animals were exposed to formaldehyde for early exposure periods of 1, 4, and 9 days and 6 weeks (Monticello et al., 1991). The second phase used osmotic minipumps for labeling with a 120-hour labeling time to quantify labeling in animals exposed for 13, 26, 52, and 78 weeks (Monticello et al., 1996). The combined pulse and continuous labeling data were expressed as one exposure time-weighted average (TWA) over all sites for each exposure concentration. α_N was calculated from these labeling data by using an approximation from Moolgavkar and Luebeck (1992). A dose-response curve for normal cell replication rates (i.e., α_N as a function of formaldehyde flux) was then calculated as shown in Figure B-16.

Upward extrapolation of normal cell division rates: The extensive labeling data collected by Monticello et al. (1996; 1991) present an opportunity to use precursor data in assessing cancer risk. However, these empirical data were used to determine α_N(flux) only for the lower flux range, 0–9,340 pmol/mm^2-hour [see Subramaniam et al. (2008) for the reasons], as shown by the solid line in Figure B-16, whereas the highest computed flux at 15.0 ppm exposure was 39,300 pmol/mm^2-hour. Therefore, Conolly et al. (2003) introduced an adjustable parameter, α_{max}, that represented the value of α_N(flux) at the maximum flux of 39,300 pmol/mm^2-hour. α_{max} was estimated by maximizing the likelihood of the two-stage model fit to the tumor incidence data. For 9,340 < flux ≤ 39,300 pmol/mm^2-hour, α_N(flux) was determined by linear interpolation from α_N(9,340) to α_{max}, as shown by the dashed line in Figure B-16.

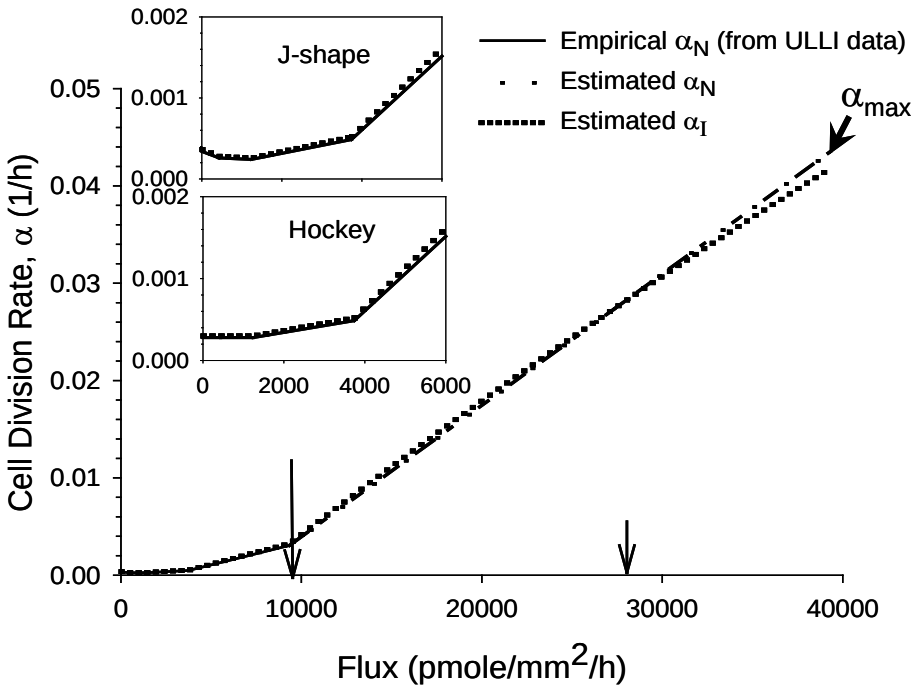


Figure B-16. Dose response of normal (α_N) and initiated (α_I) cell division rate in Conolly et al. (2003).

Note: Empirically derived values of α_N (TWA over six sites) from Table 1 in Conolly et al. (2003) and optimized parameter values from their Table 4 were used. The main panel is for the J-shaped dose response. Insets show J-shaped and hockey-stick shaped representations at the low end of the flux range. The long arrow denotes the upper end of the flux range for which the empirical unit-length labeling data are available for use in the clonal growth model. α_{max} is the value of α_N at the maximum formaldehyde flux delivered at 15 ppm exposure and estimated by optimizing model fit to the tumor incidence data. $\alpha_I < \alpha_N$ for flux greater than the value indicated by the small vertical arrow. Conolly et al. (2004, 2003) assumed $\beta_I = \alpha_N$ at all flux values.
Source: Subramaniam et al. (2008).

Division and death rates of initiated cells: The pool of cells used for obtaining the LI data in Monticello et al. (1996; 1991) consists of largely normal cells, and it may be expected that there would be increasing numbers of initiated cells at higher exposure concentrations. Because the division rates of initiated cells in the nasal epithelium, α_I, either background or formaldehyde exposed, could not be inferred from the available empirical data, Conolly et al. (2003) assumed a two-parameter function to link α_I to α_N

$$\alpha_I = \alpha_N \times \{multb - multc \times \max[\alpha_N - \alpha_{N(basal)}, 0]\} \quad \text{(B-13)}$$

where $\alpha_N \equiv \alpha_N(flux)$, $\alpha_{N(basal)}$ is the estimated average cell division rate in unexposed normal cells, and multb and multc are unknown parameters estimated by likelihood optimization against the

tumor data.[31] The value of $\alpha_{N(basal)}$ was equal to 3.39×10^{-4} hours^{-1} as determined by Conolly et al. (2003) from the raw averaged unit length labeling index data. The ratio α_I:α_N decreases with flux approximately from 1.07 to 0.96 over the flux range used in the modeling (see Figure 6 in Subramaniam et al., 2008).

Death rates of Initiated cells (β_I) are assumed to equal the division rates of normal cells (α_N) for all formaldehyde flux values, that is

$$\beta_I(\text{flux}) = \alpha_N(\text{flux}) \qquad \text{(B-14)}$$

No biological justification for these assumed relationships was provided by the authors. Conolly et al. (2003) stated that this formulation for α_I and β_I provided the best fit of the model to the tumor data.

Structure of the CIIT human model: Subsequent to the BBDR model for modeling rat cancer, Conolly et al. (2004) developed a corresponding model for humans for the purpose of extrapolating the nasal cancer risk estimated by the rat model to humans. Also, rather than considering only nasal tumors (as in the rat model), the human extrapolation model was used to predict the risk of all human respiratory tumors. The human extrapolation model is conceptually very similar to the rat model, and is based on an anatomically realistic representation of the human nasal passages in a single individual and an idealized representation of the LRT. Local formaldehyde flux to the tissue is estimated by a CFD model for humans (Kimbell et al., 2001b; Overton et al., 2001; Subramaniam et al., 1998). However, the model does not incorporate any data on human responses to formaldehyde exposure.

Rates of cell division and cell death are, with a minor modification, assumed to be the same in humans as in rats. The concentration of formaldehyde-induced DPXs in humans is estimated by scaling up from values obtained from experiments in the F344 rat and rhesus monkey.

The statistical parameters for the human model are either estimated by fitting the model to the human background data, assumed to have the same value as obtained in the rat model, or, in one case, fixed at a value suggested by the epidemiologic literature. The delay, D, is fixed at 3.5 years, based on a fit to the incidence of lung cancer in a cohort of British doctors (Doll and Peto, 1978). The two other parameters in the rat model that affect the background rate of cancer (multb and μ_{basal}) are estimated by fitting to U.S. cancer incidence or mortality data. These parameters affect the baseline values for the human α_I, μ_N, and μ_I. Because α_{max}, multfc, and KMU do not affect the background cancer rate, they cannot be estimated from the (baseline) U.S. cancer incidence rates. Therefore, in Conolly et al. (2004, 2003), α_{max} and multfc are assumed to have the same

[31]Multb and multc were equal to 1.072 and 2.583, respectively (J-shaped αN), and 1.070 and 2.515, respectively (hockey-stick shaped αN).

values in humans as in rats, and the human value for KMU is obtained by assuming that the ratio KMU:μ_{basal} is invariant across species. Thus,

$$KMU_{(human)} = KMU_{(rat)} \times \frac{\mu_{Nbasal(human)}}{\mu_{Nbasal(rat)}} \quad \text{(B-15)}$$

Evaluation of Conolly et al. (2003) Modeling of Nasal Cancer in the F344 Rat and Alternative Implementations

Table 2-24 in the dose-response section of the main document listed various issues that were evaluated by EPA pertaining to the BBDR modeling. An overview of that evaluation is first provided here, following which only the following four major issues are further elaborated: physiologically based pharmacokinetic modeling of DPXs, use of historical controls, the uncertainty and variability in the dose response for normal cell-replication rates, and sensitivity of model results to uncertainty in the kinetics of initiated cells.

Summary of Issues Evaluated in the Rat BBDR Modeling

Table B-19 summarizes model uncertainties and their impact as evaluated by EPA and points the reader to sections of this document or published manuscripts (Crump et al., 2008; Subramaniam et al., 2008; Subramaniam et al., 2007) where key uncertainties are discussed in more detail. The results in Subramaniam et al. (2007) and Crump et al. (2008) have been debated further in the literature (Conolly et al., 2009; Crump et al., 2009). Other alternatives to the CIIT biological modeling (but based on that original model) are also further explored and evaluated below.

Table B-19. Evaluation of assumptions and uncertainties in the CIIT model for nasal tumors in the F344 rat

	Assumptions, approach, and characterization of input data in model[a]	Rationale for assumption/approach	EPA evaluation	Further elaboration of evaluation
1	Steady-state flux estimates are not affected by airway and tissue reconfiguration due to long-term dosing.	Histopathologic changes not likely to be rate-limiting factors in dosimetry.	1) Thickening of epithelium and squamous metaplasia occurring at later times for the higher dose (Kimbell et al., 1997a) will reduce tissue flux. Not incorporated in model. 2) These effects will push regions of higher flux to more posterior regions of respiratory tract. Likely to affect calibration of rat model. Uncertainty not evaluated quantitatively. 3) Calibration of PBPK model for DPXs was seen to be highly sensitive to tissue thickness.	Subramaniam et al. (2008); Cohen Hubal et al. (1997); Klein et al. (2011)
2	DPX is dose surrogate for formaldehyde's mutagenic potential. DPX clearance is rapid and complete in 18 hrs.	Casanova et al. (1994).	Half-life for DPX clearance in in vitro experiments on transformed cell lines was 7 times longer than estimated by Conolly et al. (2004, 2003) and perhaps 14 times longer with normal (nontransformed) human cells. Some DPX accumulation is therefore likely. However, model calibration and dose response in rat was insensitive to this uncertainty.	Quievryn and Zhitkovich (2000); Subramaniam et al. (2007); B.2.2
3	Formaldehyde's mutagenic action takes place only while DPX's are in place.		DNA lesions may remain after DPX repair and incomplete repair of DPX can lead to mutations (Barker et al., 2005). There is some potential for formaldehyde-induced mutation after DPX clearance. Thus, it is possible that formaldehyde mutagenicity may be underrepresented in model. Could not quantitatively evaluate uncertainty (no data on clearance of secondary lesions).	Subramaniam et al. (2008);
4	Hoogenveen et al. (1999) solution method, which is valid only for time-independent parameters, is accurate enough.	Errors due to this assumption thought to be significant only at high concentration and not at human exposures.	EPA implemented a solution method valid for time-dependent parameters. Results did not differ significantly from those obtained assuming Hoogenveen et al. (1999) solutions. However, impact was not evaluated for the case where cell replication rates vary in time.	Crump et al. (2005); Subramaniam et al. (2007)

	Assumptions, approach, and characterization of input data in model[a]	Rationale for assumption/approach	EPA evaluation	Further elaboration of evaluation
5	All observed SCC tumors are rapidly fatal; none are incidental tumors.	Death is expected to occur typically within 1–2 weeks of observed tumor (personal communication with R. Conolly).	1) Overall, assumption does not impact model calibration or prediction. 2) However, because 57 animals were observed to have tumors at interim sacrifice times, EPA implementation of this model distinguished between incidental and fatal tumors. Time lag between observable tumor and time of death was significant compared to time lag between first malignant cell and observable tumor.	Subramaniam et al. (2007)
6	Historical controls from entire NTP database were lumped with concurrent controls in studies.	Large number of control animals (7,684). Intercurrent mortality was not expected to be substantial.	1) Tumor incidence in "all NTP" 10-fold higher than in "all inhalation NTP" controls. Including all NTP controls is considered inappropriate. 2) Low-dose-response curve is very sensitive to use of historical controls. 3) Large impact on parametrizations and predictions from corresponding human extrapolation model.	Subramaniam et al. (2007); Crump et al. (2008); B.2.2; Table B-21
7a	LI was derived from experimentally measured ULLI.	Derived from correlating ULLI to LI measured in same experiment.	Significant variation in number of cells per unit length of basement membrane. Spread in ULLI/LI ≈25%. Impact on risk not evaluated.	Subramaniam et al. (2008)
7b	Pulse and continuous labeling data were combined in deriving α_N from LI.	All continuous LI values were normalized by mean ratio of pulse to continuous LI for controls.	Formula used for deriving α_N from LI is not applicable for pulse labeling data. Pulse labeling is measure of number of cells in S-phase, not of their recruitment rate into S-phase; not enough information to derive α_N from pulse data. Impact on risk predictions could not be evaluated.	Subramaniam et al. (2008); B.2.2

	Assumptions, approach, and characterization of input data in model[a]	Rationale for assumption/approach	EPA evaluation	Further elaboration of evaluation
7c	To construct dose response for α_N, labeling data were weighted by exposure time (*t*) and averaged over all nasal sites (TWA). For a given exposure concentration, flux was then averaged over all nasal sites.	Site-to-site variation in LI was large and did not vary consistently with flux. No reasonable approach was available for extrapolating observed time variation in labeling in rats to humans.	1) TWA assigns low weight to early time LI values, but α_N for early time (t) is very important to the cancer process. Because pulse ULLI was used for $t < 13$ wks, impact of these ULLIs on risk could not be evaluated. 2) Time dependence in α_N derived from continuous ULLI does not significantly impact model predictions. 3) Site-to-site variation of α_N is at least 10-fold and has major impact on model calibration. Variation in tumor incidence data across sites is 10-fold. 4) Large differences in number of cells across nasal sites, so averaging over sites is problematic. 5) TWA is also problematic because histologic changes, thickening of epithelium and metaplasia occur at later times for the higher dose and would affect replication rate.	Subramaniam et al. (2008); B.2.2, Table B-22, Figures B-17 to B-26
7d	TWA α_N(flux) rises above baseline levels only at cytolethal dose. Above such dose, α_N(flux) rises sharply due to regenerative proliferation.	Variability in α_N(flux) is partly represented by also considering hockey-stick (threshold in dose) when TWA indicates J-shaped (inhibition of cell division) description of α_N(flux).	1) Uncertainty and variability in α_N were quantitatively evaluated to be large. In addition, there are several qualitative uncertainties in characterization of α_N(flux) from LI. 2) Several dose-response shapes, including a monotonic increasing curve without a threshold, were considered in order to adequately describe highly dispersed cell replication data. This has substantial impact on low dose risk.	Subramaniam et al. (2008); B.2.2, Figures B-17 to B-26

	Assumptions, approach, and characterization of input data in model[a]	Rationale for assumption/approach	EPA evaluation	Further elaboration of evaluation
8a	Dose response for α_I was obtained from α_N, assuming ratio (α_I:α_N) to be a two-parameter function of flux (see Figure B-16). Parameters were estimated by optimizing model predictions against tumor incidence data.	(α_I:α_N) was >1.0 in line with the notion of I cells possessing a growth advantage over N cells. Assumption satisfies Occam's razor principle (Conolly et al., 2009).	1) α_I:α_N in CIIT modeling is <1.0 (growth disadvantage) for higher flux values and is >1.0 only at lower end of flux range in model (Figure B-16). 2) Because there are no data to inform α_I, sensitivity of risk estimates to various functional forms was evaluated. Risk estimates for the rat were extremely sensitive to alternate biologically plausible assumptions for α_I(flux) and varied by many orders of magnitude below concentrations with observable tumors, including values lower than baseline risk. All these models described tumor incidence data and cell replication and DPX data equally well.	Subramaniam et al. (2008); Crump et al. (2008); Crump et al. (2009); B.2.2, Figures B-16, B-27, B-28
8b	Death rate of I cells is assumed equal to division rate of N cells i.e., β_I(flux) = α_N(flux).	Based on homeostasis (α_N = β_N) and assumption that formaldehyde is equally cytotoxic to N cells and I cells. Assumption satisfies Occam's razor principle (Conolly et al., 2009).	1) In general, data indicate I cells are more resistant to cytolethality and that ADH3 clearance capacity is greater in transformed cells. Therefore, β_I = α_N is a tenuous model assumption. 2) Alternate assumption, β_I proportional to α_I, was examined. Risk estimates were extremely sensitive to assumptions on β_I.	Subramaniam et al. (2008); Crump et al. (Crump et al., 2009); Crump et al. (2008); B.2.2, Figures B-27, B-28.

[a]Conolly et al. (2004, 2003).

Given the scope of issues to examine, the evaluation of the BBDR modeling as presented in Conolly et al. (2003), and in alternative approaches considered by EPA, proceeded in stages. First, the dosimetric models for formaldehyde flux and DPXs were evaluated. Confidence in the CFD modeling of formaldehyde flux has been assessed in the toxicokinetic modeling section earlier, and is not repeated here. The evaluation of PBPK models for predicting DPXs is presented below.

Second, the (Hoogenveen et al., 1999, pp. author-year) solution was replaced by one that is valid for a model with time-varying parameters [Crump et al. (2005), and tumors found at scheduled sacrifices were assumed to be incidental rather than fatal (see Table B-19 and Subramaniam et al. (2007)). Third, PBPK model-predicted weekly averaged solutions for DPX concentration levels were used instead of hourly varying solutions (see Figure 1 and Appendix A in Subramaniam et al. (2007)). The log-likelihood values and tumor probabilities remained essentially unchanged. Upon quantitative evaluation, these factors, although important from a methodological point of view, were not found to be major determinants of either calibration or

prediction of the model for the F344 rat data (Subramaniam et al., 2007). EPA evaluation first attempted to reproduce the Conolly et al. (2003) results under similar conditions and assumptions, including the assumption that tumors were rapidly fatal. Figure 2-4 of the main document shows the results from Conolly et al. (2003) and the predicted probabilities from Subramaniam et al. (2007) (source code made available by Dr. Conolly). These are compared with the best-fitting model and plotted against the Kaplan-Meier (KM) probabilities. Although the results are largely similar, there are some residual differences, and these are detailed in Subramaniam et al. (2007).

Following Georgieva et al. (2003), Subramaniam et al. (2007) used the DPX clearance rate constant obtained from in vitro data instead of the assumption in Conolly et al. (2003) that all DPXs cleared within 18 hours (Subramaniam et al., 2007). With this revision, weekly average DPX concentrations were larger than those in Conolly et al. (2003) by essentially a constant ratio equal to 4.21 (range of 4.12–4.36) when averaged over flux bin and exposure concentrations. Cancer model fits to the rat tumor incidence data using the two sets of DPX concentrations (everything else remaining the same) provided very similar parameter estimates, except that the parameter KMU_{rat} in equation B-12 was 4.23 times larger with the Conolly et al. (2003) DPX concentrations. In other words, the product KMU × DPX remained substantially unchanged. However, it is important to note that the different clearance rate does significantly impact the scale-up of the two-stage clonal growth model to the human because the parameter KMU_{human} is not estimated separately but related to KMU_{rat} (see equation B-15).

After making the above modifications, the impact of the other uncertainties in Table B-19 were examined; only three uncertainties had large impacts on the modeling of the F344 rat data. These uncertainties and the evaluation of the PBPK modeling of DPX will be discussed in more detail below:

1) evaluation and model selection of PBPK models for DPX,

2) use of historical controls,

3) uncertainty and variability in characterizing cell replication rates from the labeling data, and

4) uncertainty in model specification of initiated cell kinetics.

Physiologically based pharmacokinetic models for DPX: evaluation and model selection

The CFD modeling discussed in the toxicokinetics section models the transport of formaldehyde through the air phase to the tissue lining on the respiratory tract. While these calculations involved the specification of boundary conditions that appropriately characterize the air-tissue interface, the internal dose of formaldehyde and its reaction with tissue constituents was not explicitly modeled. Several physiologically based pharmacokinetic (PBPK) models have been developed to describe the disposition of formaldehyde in the tissue accounting for formaldehyde reaction via saturable and first order pathways that include the formation and, in some models

clearance, of DNA protein cross links (DPX) formed by formaldehyde. These models relied wholly or partly on various experimental measurements of DPX in the upper respiratory tract of the F344 rat and rhesus monkey and in the lower respiratory tract of the rhesus monkey (Casanova et al., 1994; 1991; Casanova et al., 1989), which were discussed earlier in Section A.2.2. The measurements, and subsequently the models that were based upon these data, allowed the use of formaldehyde-DPX as an internal dosimeter of inhaled formaldehyde, in particular, as a surrogate for the molecular dose associated with formaldehyde's mutagenic potential. These models are tabulated below in Table B-20.

Table B-20. PBPK models for formaldehyde-DPX

Model	Dpx data	Animal species	Human extrapolation model	Compartments and pathways	Includes air-phase formaldehyde flux?
Casanova et al. (1991)	Casanova et al. (1989); 6-hr exp; 0.3, 0.7, 2.0, 6.0, 10 ppm Casanova et al. (1991); 6-hr exp; 0.7, 2.0, 6.0 ppm	F344 rat Rhesus monkey	No	Single well-stirred compartment. Saturable & 1st order metabolism, 1st order DPX formation but not clearance.	No
Heck & Casanova (1994)	Casanova et al. (1994); 0.7, 2, 6, 15 ppm preexposed + naïve groups	F344 rat	No	Similar to Casanova et al. (1991). Included effects of preexposure, induction of hyperplasia at conc > 6 ppm.	No
Cohen Hubal et al. (1997)	Casanova et al. (1989) above + Casanova (1994); 3-hr exp; 0.7, 2.0, 6.0, 15 ppm	F344 rat	No	Casanova (1991) model+air-phase transport+ 1st order DPX clearance. Predicted DPX in a more localized region based on model calibrated over whole nose	Yes (Kimbell et al., 1997a)
Conolly et al. (2000)	Casanova et al. (1989) above + Casanova (1994); 3-hr exp, 0.7, 2.0, 6.0, 15 ppm Casanova et al. (1991); 6-hr exp; 0.7, 2.0, 6.0 ppm	F344 rat Rhesus monkey	Yes	Similar to Cohen Hubal et al. (1997). Derived allometric rule based on rat and rhesus model to develop human extrapolation model	Yes (Kimbell et al., 2001b)
Georgieva et al. (2003)	Casanova et al. (1989) above +	F344 rat	No	Multilayer tissue compartment, epithelia of varying thickness. Saturable & 1st order metabolism, 1st	Yes, (Kimbell et al., 2001b)

Model	Dpx data	Animal species	Human extrapolation model	Compartments and pathways	Includes air-phase formaldehyde flux?
	Casanova (1994) 3 hr exp, 0.7, 2.0, 6.0, 15 ppm			order DPX formation & clearance, clearance rate derived from in vitro data	
Franks et al. (2005)	Did not use data on DPX or formaldehyde levels for calibration. Parameter values from other models were used.	Model developed for humans		Continuous distribution of formaldehyde across mucous, epithelial & blood perfused submucosal layers; diffusional transport of formaldehyde through mucous layer; Saturable & 1st order metabolism, 1st order DPX formation but not clearance. Model evaluated systemic transport of formaldehyde.	No
Subramaniam et al. (2007)	Casanova et al. (1989) above + Casanova (1994) 3 hr exp, 0.7, 2.0, 6.0, 15 ppm.	F344 rat	No	Saturable & 1st order metabolism, 1st order DPX formation & clearance, clearance rate derived from in vitro data	Yes, (Kimbell et al., 2001b)

In addition, Klein et al. (2011) used Conolly et al. (2000) as a case study to demonstrate approaches for uncertainty analyses of PBPK modeling for situations involving limited time course data. Of the models in Table B-20, clearance of DPX by repair processes was not considered in Casanova et al. (1991), Heck and Casanova (1994) and Franks et al. (2005), and only Conolly et al. (2000) extended their animal PBPK model to develop a corresponding model for the human. The Conolly et al. (2000) modeling presents other features that are useful in the context of modeling formaldehyde dose response. Their PBPK modeling of DPX kinetics explicitly incorporates regional formaldehyde dosimetry in the nasal lining by using results from CFD modeling of airflow and gas uptake. Furthermore, results from their models were used as input to biologically based cancer dose-response (BBDR) modeling developed by the same authors. Because of these reasons, EPA evaluated the Conolly et al. (2000) PBPK effort, following which it was modified (see Appendix A in Subramaniam et al. (2007)) and used in EPA's dose-response assessment. The Conolly et al. (2000) model is first described below.

In earlier risk assessment efforts by Hernandez et al. (1994) and Casanova et al. (1991), the average DPX concentration was considered a surrogate tissue dose metric for the area-under-the-curve (AUC) of the reactive formaldehyde species. Conolly et al. (2003) assigned a more specific role for DPXs, treating local DPX concentration as a dose surrogate indicative of the intercellular concentration of formaldehyde leading to formaldehyde-induced mutations. These authors indicated that it was not known whether DPXs directly induced mutations (Conolly et al., 2003;

Merk and Speit, 1998). The Conolly et al. (2000) model consists of a single well-mixed compartment for the nasal lining as follows:

1) Formaldehyde flux to a given region of the nasal lining is provided as input to the modeling and is obtained in turn as the result of a CFD model. This flux is defined as the amount of formaldehyde delivered to the nasal lining per unit time per unit area per ppm of concentration in the air in a direction transverse to the airflow. It is locally defined as a function of location in the nose and the inspiratory flow rate and is linear with exposure concentration.

2) The clearance of formaldehyde from the tissue is modeled as a saturable pathway representing enzymatic metabolism of formaldehyde primarily by formaldehyde dehydrogenase (involving Michaelis-Menten parameters Vmax and K_m); a separate first-order pathway, which is assumed to represent the intrinsic reactivity of formaldehyde with tissue constituents (rate constant k_f); and a first-order binding to DNA that leads to DPX formation (rate constant k_b).

3) The clearance or repair of DPX is modeled as a first order process (rate constant k_{loss}).

DPX concentrations were estimated from a study by Casanova et al. (1994) in which rats were exposed 6 hours/day, 5 days/week, plus 4 days for 11 weeks to filtered air (naive) or to 0.7, 2, 6, or 15 ppm (0.9, 2.5, 7.4, or 18 mg/m^3) formaldehyde (preexposed). On the 5th day of the 12th week, the rats were then exposed for 3 hours to 0, 0.7, 2, 6, or 15 ppm 14C-labeled formaldehyde (with preexposed animals exposed to the same concentration as during the preceding 12 weeks and 4 days). The animals were sacrificed and DPX concentrations determined at two sites in the nasal mucosa. Conolly et al. (2000) used these naive rat data to develop a PBPK model that predicted the time-course of DPX concentrations as a function of formaldehyde flux at these sites.[32]

Casanova et al. (1994) observed that the DPX concentrations measured in the preexposed animals (exposed for 11.5 weeks) were not significantly higher than those in naïve (air-exposed control) animals in which there was no significant DPX accumulation. This was interpreted to mean that DPX repair is rapid enough to completely eliminate the DPX formed in a single 6-hour exposure by the beginning of the next day. Based on this observation, Conolly et al. (2000) assumed a value of 6.5×10^{-3} minute^{-1} for *kloss*, the first-order rate constant for the clearance (repair) of DPXs, such that the DPXs predicted at the end of a 6-hour exposure to 15 ppm were reduced to exactly the detection limit for DPXs in 18 hours.

Uncertainties in PBPK Modeling of the Rat and Rhesus DPX Data

The above assumption of rapid DPX repair in Conolly et al. (2000) appears to be questionable on three grounds. First, in vitro data from three human cell lines indicated a much slower clearance, with an average *kloss* of 9.24×10^{-4} minute^{-1} (Quievryn and Zhitkovich, 2000).

[32]Subramaniam et al. (2007) who also used the same data verified that they were on naïve rats; however, Conolly et al. (2000) state that they used data on preexposed rats.

While the in vitro data can be uncertain because these cells were transformed and immortalized, it appears that DPX repair in normal cells would be even slower. When nontransformed freshly purified human peripheral lymphocytes were used instead, the half-life for DPX repair was about 50% longer than in the cultured cells (Quievryn and Zhitkovich, 2000).

Second, Subramaniam et al. (2007) reexamined the Casanova et al. (1994) data for their PBPK modeling and concluded that the experimental results in Casanova et al. (1994) were consistent with the smaller experimental value of *kloss* indicated by the Quievryn and Zhitkovich (2000) data. Subramaniam et al. (2007) found a significantly decreased (≈ 40%) level of DPXs in the high tumor regions of preexposed animals relative to naive animals at 6 and 15 ppm. This was accompanied by a substantial increase in weight of the tissues dissected from those regions indicating a thickening of the tissues as is to be expected from metaplastic transformation of normal tissue to the squamous type due to formaldehyde toxicity. However, after testing the outcome of changing the tissue thickness in the PBPK model for DPXs, it was apparent to these authors that such a change alone could not account for the dramatic reduction in DPX levels after preexposure, even with the higher value of *kloss* used by Conolly et al. (2000). Because Vmax was found to be very sensitive to tissue thickness (as also noted by others; (Klein et al., 2011; Georgieva et al., 2003; Conolly et al., 2000)), Subramaniam et al. (2007) increased the value of Vmax with exposure (in a tissue region- and dose-specific manner) and found that it was possible to explain the naïve versus preexposed data of Casanova et al. (1994) with the 7-fold lower value of *kloss*. This was consistent with the hypothesis of either an induction in the activity of enzymes that remove formaldehyde (aldehyde- and formaldehyde dehydrogenase) or other changes in the biochemical properties of highly exposed tissue.

Third, the value for *kloss* used by Conolly et al. (2000) was inferred indirectly from measurements made at only two time points where significant changes in the tissue had occurred. On account of these reasons, Subramaniam et al. (2007) considered the use of the lower value for *kloss* from in vitro observations to be more appropriate. The same lower value of *kloss* was also used by Georgieva et al. (2003). Consequently, Subramaniam et al. (2007) reimplemented and reoptimized the Conolly et al. (2000) model with this modification and obtained a good fit to the acute DPX data. The reimplemented model is used in this assessment. Both models provide good similar fits to the DPX data gathered from different regions of the nose immediately after single 3.0-hour and 6.0-hour acute exposures.

Sensitivity to use of historical controls

Use of historical controls: Conolly et al. (2003) combined the historical controls arising from the entire NTP database of bioassays. Tumor and survival rates in control groups from different NTP studies are known to vary due to genetic drift in animals over time and differences in laboratory procedures, such as diet, housing, and pathological procedures (Haseman, 1995; Rao et al., 1987). In order to minimize extra variability when historical control data are used, the current NTP practice is to limit the historical control data, as far as possible, to studies involving the same

route of exposure and to use historical control data from the most recent studies (Peddada and Kissling, 2006).

Bickis and Krewski (1989) analyzed 49 NTP long-term rodent cancer bioassays and found a large difference in determinations of carcinogenicity, depending on the use of historical controls with concurrent control animals. The historical controls used in the CIIT modeling controls came from different rat colonies and from experiments conducted in different laboratories over a wide span of years, so it is clearly problematic to assume that background rates in these historical control animals are the same as those in the concurrent control group. There are considerable differences among the background tumor rates of SCCs in all NTP controls (13/7,684 = 0.0017), NTP inhalation controls (1/4,551 = 0.0002), and concurrent controls (0/341 = 0.0). The rate in all NTP controls is significantly higher than that in NTP inhalation controls (p = 0.01, Fisher's exact test). Given these differences, the inclusion of any type of historical controls is problematic and is thought to have limited value if these factors are not controlled for (Haseman, 1995).

Influence of historical controls on model calibration and on human model: To investigate the effect of including historical controls in the CIIT model, the analyses in Subramaniam et al. (2007) were conducted by using the following sets of data for controls (the fraction of animals with SCCs is denoted in parentheses): a) only concurrent controls (0/341), b) concurrent controls plus all the NTP historical control data used by Conolly et al. (2003) (13/8,031), c) concurrent controls plus data from historical controls obtained from NTP inhalation studies (1/4,949) (National Toxicology Program (NTP), 2005).[33]

The results of the evaluation are shown in Table B-21. For these analyses, the same normal cell replication rates and the same relationship, equation B-13, between initiated cell and normal cell replication rates as used in Conolly et al. (2003) were used. In all cases, weekly averaged values of DPX concentrations were used. Model fits to the tumor incidence data were similar in all cases to that shown in Figure 2-4 [see Subramaniam et al. (2007) for a more complete discussion]. The biggest influence of the control data was seen to be on the estimated basal mutation rate in rats, $\mu_{Nbasal(rat)}$, which, in turn, influences the estimated mutation effect in humans through equation B-15. α_{max} was also seen to be a sensitive parameter and is discussed later. See Subramaniam et al. (2007) for other parameters in the calibration.

[33]Three animals in the inhalation historical controls were diagnosed with nasal SCC. Of these, two of the tumors were determined to have originated in tissues other than the nasal cavity upon further review (Dr. Kevin Morgan and Ms. Betsy Gross Bermudez, personal communication). These two tumors, therefore, were not included on the advice of Dr. Morgan. See Subramaniam et al. (2007) for more details.

Table B-21. Influence of control data in modeling formaldehyde-induced cancer in the F344 rat

Case	A	D	B	E	C	F
Control animals (combined with concurrent controls)	**All NTP historical[a]**	**All NTP historical[a]**	**NTP inhalation historical[a]**	**NTP inhalation historical[a]**	**Concurrent only[a]**	**Concurrent only[a]**
Cell replication dose response	**J shape**	**Hockey stick**	**J shape**	**Hockey stick**	**J shape**	**Hockey stick**
Log-likelihood	−1,692.65	−1,693.68	−1,493.21	−1,493.35	−1,474.29	−1,474.29
μNbasal	1.87×10^{-6}	2.12×10^{-6}	7.32×10^{-7}	9.32×10^{-7}	0.0	0.0
KMU	1.12×10^{-7}	0.0	6.84×10^{-7}	6.18×10^{-7}	1.20×10^{-6}	1.20×10^{-6}
KMU:μNbasal	0.06 (0.0, 0.40)	0.0 (0.0, 0.25)	0.94 (0.26, 6.20)	0.66 (0.2, 5.20)	∞ (0.42, ∞)	∞ (0.41, ∞)
αmax	0.045 (0.029, 0.045)	0.045 (0.029, 0.045)	0.045 (0.026, 0.045)	0.045 (0.027, 0.045)	0.045 (0.027, 0.045)	0.045 (0.027, 0.045)

[a]Values in parentheses denote lower and upper 90% confidence bounds.
Source: Adapted from Subramaniam et al. (2007).

The ratio KMU:μ_{Nbasal} is of particular interest because extrapolation to human in Conolly et al. (2004) assumed its invariance as given by equation B-15. Now, μ_{Nbasal} in the human is estimated independently by fitting a scaled-up version of the two-stage model to human baseline rates of tumor incidence. Thus, a decrease in the value of μ_{Nbasal} estimated in the rat modeling increases the formaldehyde-induced mutational effect in the human.

The MLE of KMU_{rat}:$\mu_{Nbasal(rat)}$ is zero in (Conolly et al., 2003). However, in the various cases examined in Subramaniam et al. (2007) it takes a range of values from 0 to 0.9 mm^3/pmol and undefined (or infinite, when $\mu_{Nbasal} = 0$). The 95% upper confidence bound on this ratio ranges from 0.25–6.2 [these values would be four times larger had the Conolly et al. (2003) DPX concentrations been used] to infinite. Thus, the extrapolation to human risk by using the approach in Conolly et al. (2004) becomes particularly problematic when only concurrent controls are used, because then the mutational contribution to formaldehyde-induced risk in humans becomes unbounded. This issue will be discussed again toward the end of the discussion on historical controls.

It may be noted, however, that absence of tumors in the limited number of concurrent animals does not imply that the calculation will necessarily predict a zero background probability of tumor (i.e., a parameter estimate of $\mu_{Nbasal} = 0$). Nonetheless, when $\mu_{Nbasal} = 0$, an upper bound for μ_{Nbasal} using the concurrent controls could be inferred. Accordingly, the 90% statistical lower confidence bound on the ratio KMU:μ_{Nbasal} is also reported in Table B-21. Such a value would of course provide a lower bound on risk by using this model and, therefore, would not be conservative.

Conolly et al. (2003) estimated KMU to be zero for both their hockey-stick and J-shaped dose-response models for cell replication. However, the estimate for the coefficient KMU [obtained

using the solution of Crump et al. (2005)] is zero only for the case of the model with the hockey-stick curve for cell replication and with control data as used by Conolly et al. (2003). It is positive in all other cases and statistically significantly so in all cases in which either NTP inhalation control data or concurrent controls were used. With concurrent controls only and the J-shaped cell replication model, the MLE estimate for KMU (1.2×10^{-6}) is larger than the statistical upper bound obtained by Conolly et al. (2003) (8.2×10^{-7}). The estimate would be about 4.2 times larger had the Conolly et al. (2003) DPX model been used.

Influence of historical controls on dose-response curve: Subramaniam et al. (2007) showed that inclusion of historical controls had a strong impact on the tumor probability curve below the range of exposures over which tumors were observed in the formaldehyde bioassays. As shown there, the MLE probabilities for occurrence of a fatal tumor at exposure concentrations below 6 ppm were roughly an order of magnitude higher when all the NTP historical controls were used, compared with MLE probabilities predicted when historical controls were drawn only from inhalation bioassays, and many orders of magnitude higher than MLE probabilities predicted when only concurrent controls were used in the analysis. (Note that this comparison should not be inferred to apply to upper bound risk estimates because there were many fewer concurrent than historical controls, so error bounds could be much larger in the case where concurrent controls were used.)

However, as shown by these authors, model fits to the tumor data in the 6–15 ppm exposure concentration range were qualitatively indifferent to which of these control data sets was used. This observation emphasizes the statistical aspect of the CIIT modeling—that significant interplay among the various adjustable parameters allows the model to achieve a good fit to the tumor incidence data independent of the control data used. On the other hand, the results in Subramaniam et al. (2007) show that changes in the control data affect parameter KMU, resulting in significantly different tumor predictions at lower exposure concentrations. Therefore, the strong influence of using all the NTP historical controls on the low-dose region of the time-to-tumor curves presented in Subramaniam et al. (2007) suggests that large uncertainties may arise in extrapolating to both human and rat (in the low-dose region) from such considerations alone.

A crucial point needs to be noted with regard to the use of inhalation NTP historical controls (i.e., cases B and E) in the two-stage clonal growth modeling. The single relevant tumor in the NTP inhalation studies came from the very first NTP inhalation study, dated 1976, and the animals in this study were from Hazelton Laboratories, whereas the concurrent animals were all from Charles River Laboratories. Similar problems arise with inclusion of several other NTP inhalation studies. As mentioned before, genetic and other time-related variation can lead to different tumor and survival rates, and in general it is recommended that use of historical controls be restricted to the same kind of bioassays and to studies within a 5–7 year span of the concurrent animals (Peddada et al., 2007). Thus, it is problematic to assume that the tumor in the 1976 NTP study is representative of the risk of SCCs in the formaldehyde bioassays. Even if it were appropriate to consider the 1976

study, this leads to the unstable situation in which the only piece of data that might keep the model predictions of human risk bounded is a single tumor found among several thousand rats from NTP bioassays (Crump et al., 2008). In summary, although it can be argued that the rate of SCCs among the controls in the rat bioassay is probably not zero, it is also problematic to assume that this rate can be adequately represented by the background rate in NTP historical controls or even in NTP inhalation historical controls.

Effect of historical controls on modeling inferences regarding mode-of-action:

Subramaniam et al. (2007) also examined the contribution of the DPX component (which represents the directly mutagenic potential of formaldehyde in the model) to the calculated tumor probability, choosing for their case study the optimized models that use the NTP inhalation control data. In the range of exposures where tumors were observed (6.0–15.0 ppm), the DPX term was found to be responsible for 58–74% of the added tumor probability. Below 6.0 ppm the estimated DPX contribution was extremely sensitive to whether the hockey-stick shape or J-shaped was used to characterize the dose response for cell replication, and varied between 2% and 80%.

Several formaldehyde risk assessment efforts and papers have argued based on the CIIT BBDR cancer modeling that the direct mutations induced by formaldehyde are relatively irrelevant compared to the importance of cytotoxicity-induced cell proliferation in explaining the observed tumorigenicity in rodent bioassays (Conolly et al., 2004; Slikker et al., 2004; Bogdanffy et al., 2001; Bogdanffy et al., 1999). The reanalyses in Subramaniam et al. (2007) (in particular, the results in the above paragraph) indicate that, if the CIIT mathematical modeling were used to inform this debate, it would in fact suggest the contrary—that a large contribution from formaldehyde's mutagenic potential may be needed to explain formaldehyde carcinogenicity. It may also be noted that because the BBDR modeling estimates the constant of proportionality relating DPX levels to formaldehyde-induced mutation by fitting to the steeply rising tumor incidence data, EPA's uncertainty analysis of results derived from the modeling reflects [model] uncertainty associated with a mutagenic mode of action.

Characterization of uncertainty-variability in cell replication rates

Dose-response for normal cell division rate as used in model

Monticello et al. (1996; 1991) used unit length labeling index (ULLI) to quantify cell replication within the respiratory epithelium. ULLI is a ratio between a count of labeled cells and the corresponding length (in millimeters) of basal membrane examined, whereas the per-cell labeling index (LI) is the ratio of labeled cells to all epithelial cells, in this case, along some length of basal membrane and its associated layer of epithelial cells. Monticello et al. (1996; 1991) published ULLI values averaged over replicate animals for each combination of exposure concentration, exposure time, and nasal site. These values are plotted in Figure B-17.

To use the ULLI data in clonal growth modeling, ULLI needed to be related to LI, and thereby to cell replication rate (α_N) of normal cells. Conolly et al. (2003) adopted the following procedure in using these values:

1) The injection labeled ULLI data were first normalized by the ratio of the average minipump ULLI for controls to the average injection labeled ULLI for controls.

2) Next, these ULLI average values were weighted by the exposure times in Monticello et al. (1996; 1991) and averaged over the nasal sites. Thus, the data were combined into one TWA for each exposure concentration.

3) LI was linearly related to the measured ULLI by using data from a different experiment (Monticello et al., 1990) where both quantities had been measured for two sites in the nose.

4) Cell replication rates of normal cells (α_N) were then calculated as $\alpha_N = (-0.5/t)\log(1 - LI)$ (Moolgavkar and Luebeck, 1992), where LI is the labeling index and t is the period of labeling.

5) This was repeated for each exposure concentration of formaldehyde, resulting in one value of α_N for each exposure concentration.

6) Correspondingly, for a given exposure concentration, the steady-state formaldehyde flux into tissue, computed by CFD modeling, was averaged over all nasal sites. Thus, the α_N(flux) constructed by Conolly et al. (2003) consisted of a single α_N and a single average flux for each of six exposures.

This yielded a J-shaped dose-response curve for cell replication (when viewed on a nontransformed scale for α_N), as shown in Figure B-16 for the full range of flux values used in their modeling. The authors also considered a hockey-stick threshold representation of their J-shaped curve for α_N in order to make a health-protective choice, and the differences between the two can be seen from the insets in the Figure. In these curves, the cell replication rate is less than or the same as the baseline cell replication rate at low formaldehyde flux values. The shape of the dose-response curve for cell replication as characterized in Conolly et al. (2003) is seen as representing regenerative cell proliferation secondary to the cytotoxicity of formaldehyde (Conolly, 2002). Considerable uncertainty and variability, both quantitative and qualitative, exist in the use and interpretation of these labeling data for characterizing a dose response for cell replication rates. The primary issues are discussed here. Unlike the preceding sections, these have largely not been published elsewhere, so more details are provided.

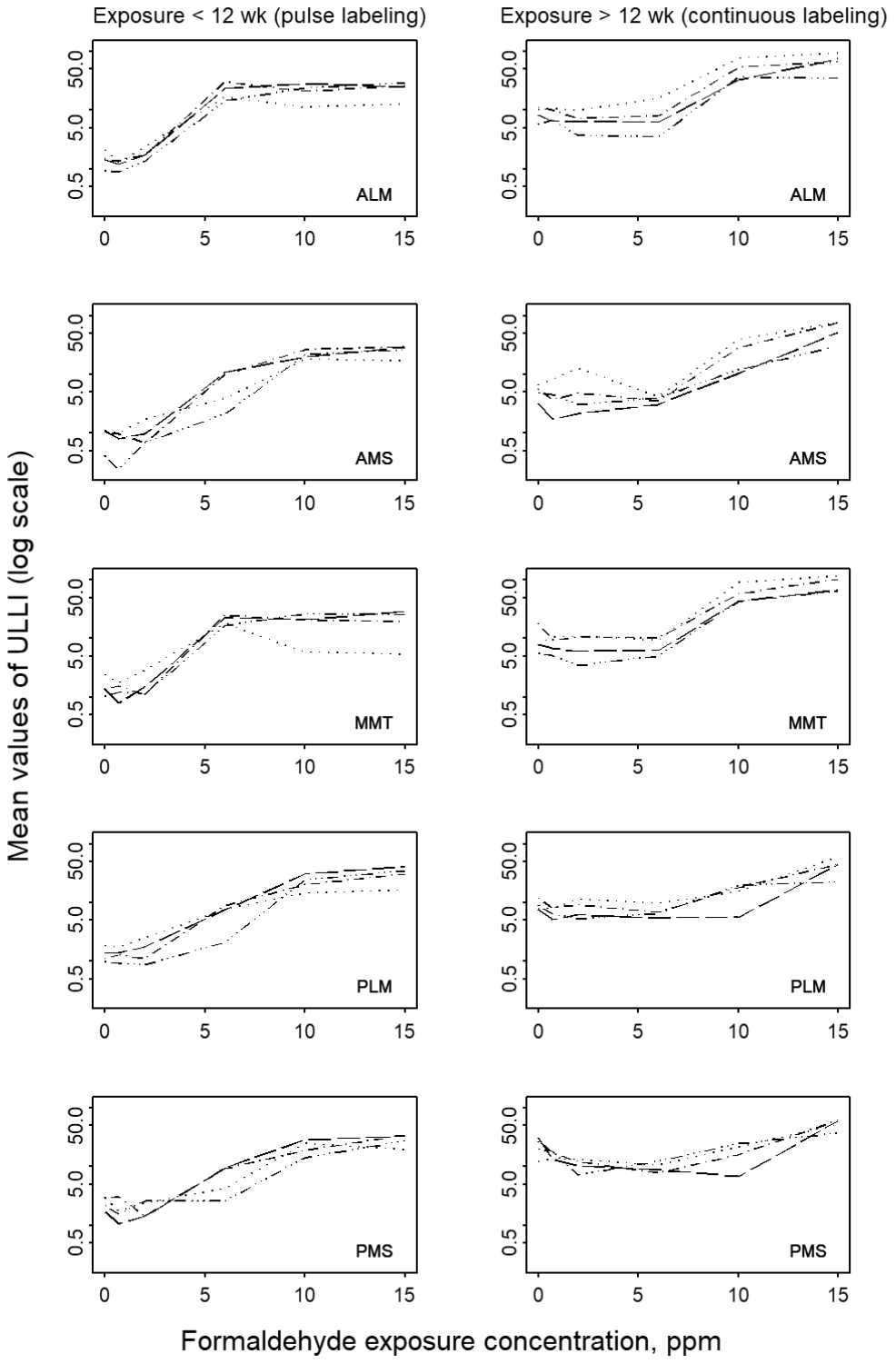


Figure B-17. ULLI data for pulse and continuous labeling studies.

Note: Data are from pulse labeling study, left-hand side, at 1–42 days of exposure and from the continuous-labeling study, right-hand side, at 13–78 weeks of exposure for five nasal sites ALM, AMS,

MMT, PLM, and posterior mid septum [PMS]). Within each graph, lines with more breaks correspond to shorter exposure times. Data source: Monticello et al. (1996; 1991).

Time variability in labeling data

Short-time exposure effects on cell replication: Figure B-17 shows the site and time variation in the raw unit-length labeling index (ULLI) data for 1 day to 78 weeks of exposure duration. The dose-response for ULLI is quite different between the "early time" (left panel) and "later time" (right panel) and these early time effects may be quite important to the cancer modeling. At the earliest times in the left panel, the data show an increased trend in labeling at 2 ppm for the sites anterior lateral meatus (ALM), anterior medial septum (AMS), posterior lateral meatus (PLM), and medial maxilloturbinate (MMT) relative to control.

The early times would be important if, say, repeated episodic exposures were considered, where adequate time has not elapsed for adaptive effects to take place. Such an exposure scenario may be the norm in the human context. In the cancer modeling in Conolly et al. (2003), because the LI was weighted by exposure time, the contribution of the early time labeling data is minimized.

Uncertainty due to combining pulse and continuous labeled data: The formula used for obtaining α_N from LI in Conolly et al. (2003) was due to Moolgavkar and Luebeck (1992) who derived this formula for continuous LI, cautioning that it is not applicable for pulse labeled data. However, Conolly et al. (2003) applied this formula to the injection (pulse) labeled data also. Such an application is problematic because 2-hour pulse labeled data represent the pool of cells in S-phase rather than the rate at which cells are recruited to the pool, and because the baseline values of α_N obtained in this manner from both data sets differ considerably. As such, we are not aware of any reasonable manner to derive cell replication rates from these pulse data without acquisition of data at additional time points. Because of these problems in incorporating the pulse-labeled data, further quantitative analysis of cell replication rates is restricted in this document to the continuous labeled data (Monticello et al., 1996), which do not include measurements made before 13 weeks of exposure. It is unfortunate that the continuous labeled data do not include any early measurements.

Site and time variability in derived cell replication rate

In the remainder of this section, the factors that are considered in order to represent the uncertainty and variability in the cell replication data when developing alternate dose-response curves for α_N(flux) will be elaborated.

The ULLI data for individual animals were provided by CIIT, which were transformed to LI values using the linear relationship from step 3 above. For these replicate data, cell replication rates of normal cells (α_N) were then calculated as $\alpha_N = (-0.5/t)\log(1 - LI)$ as in Step 4. Figure B-18 (adapted from adapted from Subramaniam et al., 2008) shows the variability in α_N due to replicated animals, exposure times, and nasal sites in the continuous labeled data obtained by Monticello et al. (1996). In this figure, log α_N versus site-specific flux are plotted for six sites and four exposure times for four to six replicate animals in each case. (The mean ULLI over these replicates were

shown in Figure B-17 for each site and time as a function of exposure concentration.) It needs to be noted that these nasal sites differ considerably in the number of cells estimated at these locations as shown in Table B-22. Each point in Figure B-18 represents data from a single site for a single animal at a given time. For comparison, the time weighted and site averaged α_N(flux) in Conolly et al. (2003) is also plotted in this figure at their averaged flux values (filled red circles). For flux >9,340 pmol/mm^2-hour, Conolly et al. (2003) extrapolated this empirically derived α_N(flux) by using a scheme discussed in the section on model structure and calibration in B.2.2. The curves shown connecting the filled circles in the figure represent their linear interpolation (long dashes) among the six points. Their linear extrapolation for flux value >9,340 pmol/mm^2-hour is also shown (short dashes). Note that the linear interpolation and extrapolation are shown transformed to a logarithmic scale in this plot.

As discussed, the raw labeling data plotted in Figure B-17 indicates considerable temporal variability. In Figure B-19, fitted dose-response curves showing $\log_{10}(\alpha_N)$ versus flux with simultaneous confidence limits separately for each time point for two of the largest sites in Table B-22 (ALM and PLM) are plotted for the continuous labeled data. Note that flux levels are different at each site. Simple polynomial models in flux (as a continuous predictor), with time included as a factor (i.e., a class or indicator variable, τ_i representing the effect of the i^{th} time) were used as follows:

$$\log(\alpha_N) = a + b \times flux + c \times flux^2 + d \times flux^3 + \tau_i \qquad \text{(B-16)}$$

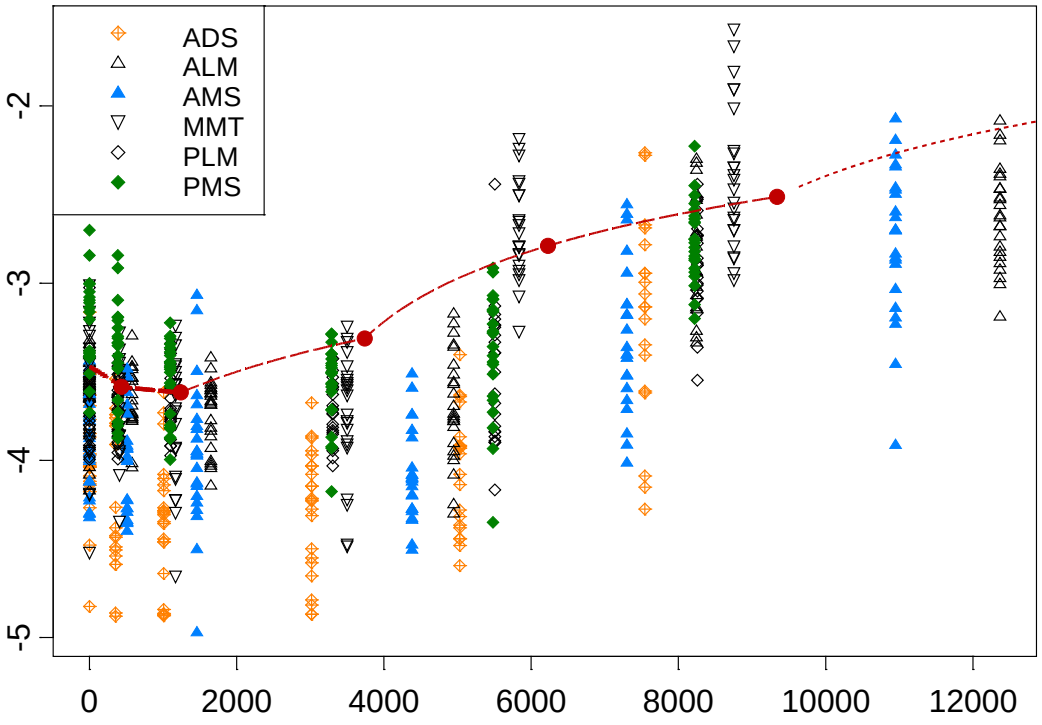


Figure B-18. Logarithm of normal cell replication rate α_N versus formaldehyde flux (in units of pmol/mm²-hr) for the F344 rat nasal epithelium.

Note: Values were derived from continuous unit length labeled data obtained by Monticello et al. (1996) for four to six individual animals at all six nasal sites (legend, sites as denoted in original paper) and four exposure durations (13, 26, 52, 78 weeks). Each point represents a measurement for one rat, at one nasal site, and at a given exposure time. Filled red circles: α_N(flux) used in Conolly et al. (2003) plotted at their averaged flux values (see text for details). Long dashed lines: their linear interpolation among points. Short dashed line: their linear extrapolation for flux values 9,340 to 39,300 pmol/mm²-hr (see Figure B-16 for full range of extrapolation). Linear interpolation/extrapolation is shown with *y*-axis transformed to logarithmic scale.
Source: Subramaniam et al. (2008).

Table B-22. Variation in number of cells across nasal sites in the F344 rat

Nasal site	No. of cells
Anterior lateral meatus	976,000
Posterior lateral meatus	508,000
Anterior mid septum	184,000
Posterior mid septum	190,000
Anterior dorsal septum	128,000
Anterior medial maxilloturbinate	104,000

Note: Mean number of cells in each side of the nose of control animals.
Source: Monticello et al. (1996).

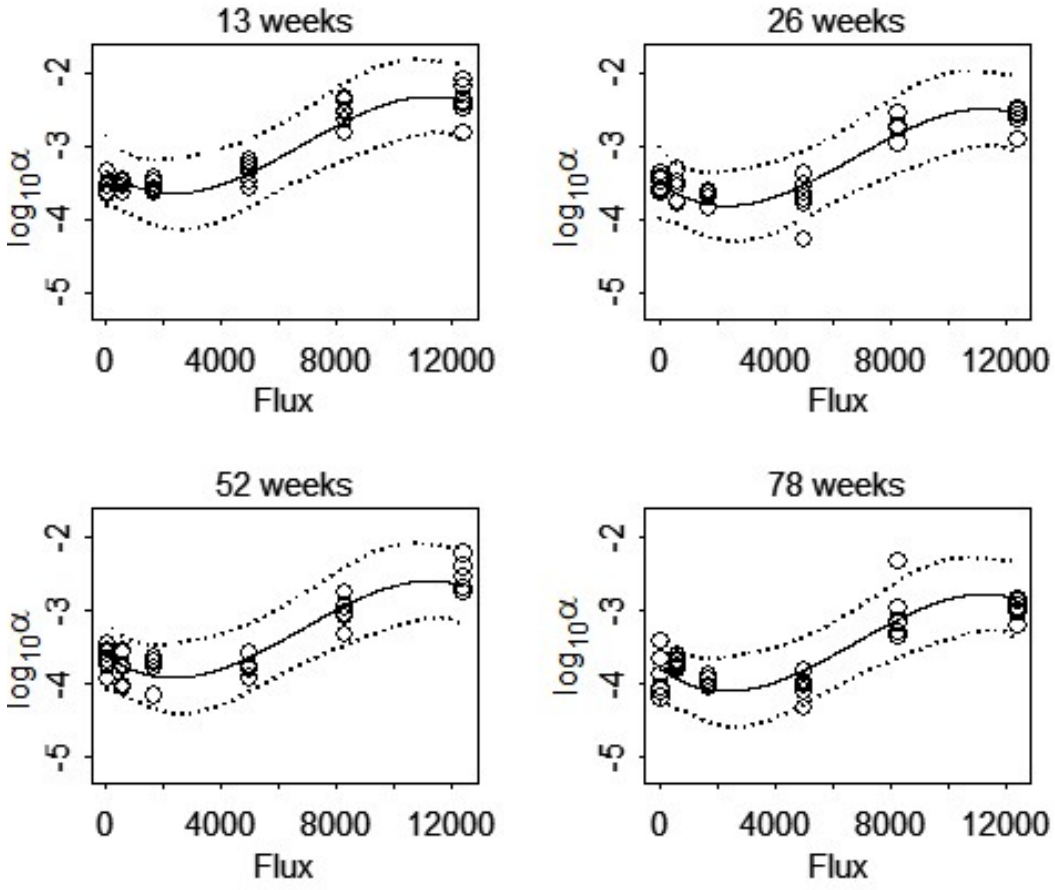


Figure B-19. Logarithm of normal cell replication rate versus formaldehyde flux with simultaneous confidence limits for the ALM.

Source: Subramaniam et al. (2008).

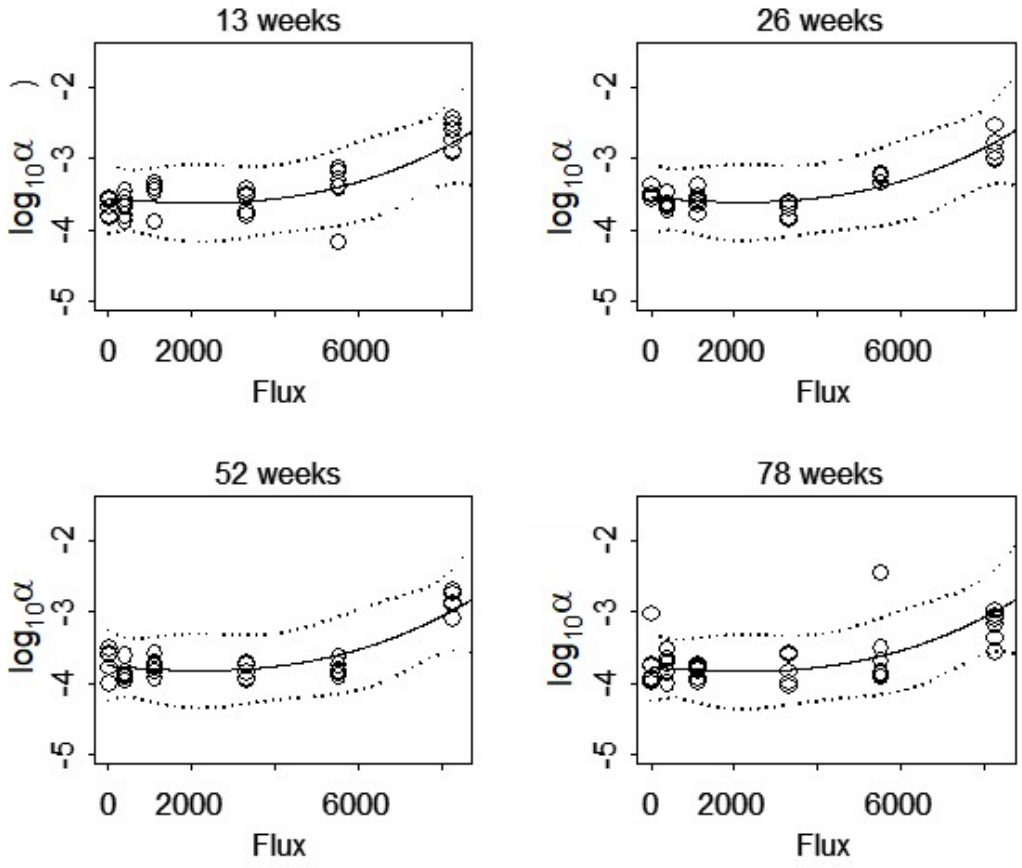


Figure B-20. Logarithm of normal cell replication rate versus formaldehyde flux with simultaneous confidence limits for the PLM.

Source: Subramaniam et al. (2008).

The variability considered is that among animals and any measurement error as well as any other design-related components of error. Simultaneous 95% confidence limits for $\log(\alpha_N)$ were produced using Scheffe's method (Snedecor and Cochran, 1980). These 95% confidence limits span a range of 0.96 in $\log10(\alpha_N)$, or nearly a 10-fold range in median α_N. There is additional dispersion in these data that does not appear in Figures B-18 to B-20 for α_N, derived using the mean value of ULLI:LI; due to variation in the number of cells per mm basement membrane, the ratio of ULLI:LI had a spread of approximately ±25% (0.45 to 0.71, mean 0.60) among the eight observations considered in Monticello et al. (1990). Thus:

1) As suggested by Table B-22, and Figures B-19 and B-20, the shape of α_N(flux) in Conolly et al. (2003) is likely to be very sensitive to how α_N is weighted and averaged over site and time.

2) Averaging of sites could significantly affect model calibration because of substantial nonlinearity in model dependence on α_N at the 10 and 15 ppm doses associated with high cancer incidence.

3) Monticello et al. (1996) found a high correlation between tumor rate and the ULLI weighted by the number of cells at a site. Therefore, considering these factors while regressing α_N against tissue dose would be important in the context of site differences in tumor response.

4) Histologic changes and thickening occur in the nasal epithelium over time in the higher dose groups (Morgan, 1997), factors that are likely to affect estimates of local formaldehyde flux, uptake, and replication rates (Subramaniam et al., 2008).

It is clear from Figures B-17, B-19 and B-20 that the time dependence in cell replication is significant. It would also be useful to examine if this time dependence affects the results of the time-to-tumor modeling and if early temporal changes in replication rate are important to consider because of the generally cumulative nature of cancer risk. The time window over which formaldehyde-induced cancer risk is most influenced is not known, but the time weighting used by Conolly et al. (2003) assigns a relatively low weight to labeling observed at early times compared with those observed at later time points. Finally, initiated cells are likely to be replicating at higher rates than normal cells as evidenced in several studies on premalignant lesions (Coste et al., 1996; Dragan et al., 1995; Rotstein et al., 1986). Therefore, LI data as an estimator of normal cell replication rate would be most reliable at early times when the mix of cells sampled include fewer preneoplastic or neoplastic cells.

Given the above uncertainties and variability not characterized in CIIT (CIIT, 1999) or in Conolly et al. (2003), it is important to examine whether additional dose-response curves that fit the cell replication data reasonably well have an impact on estimated risk. Such sensitivity analyses are carried out in the sections that follow.

Alternate dose-response curves for cell replication

Clearly, a large number of alternative α_N(flux) can be developed. In conjunction with the other uncertainties, mainly the use of control data and alternative model structures for initiated cell kinetics, the number of plausible clonal growth models to be exercised soon require a prohibitively large investment of time. Therefore, detailed analyses were restricted to a select set of biologically plausible choices of curves for α_N(flux), which would allow the identification of a range of plausible risk estimates (MLEs and statistical bounds).

Six alternative equations for α_N were developed by regression analysis of the Monticello et al. (1996) ULLI data. The replicate data corresponding to the summary data presented in this paper were kindly provided to EPA by CIIT for further analyses. In each of these equations, α_N is expressed as a function of formaldehyde flux to nasal tissue (pmol/mm^2-hour) and, in one equation (see equation B-22) that explored time-dependence, the duration of exposure to formaldehyde in weeks. All the graphs use flux/10,000 for the *x*-axis, and the *y*-axis expresses $\log_{10} \alpha_N$.

One source of uncertainty in the cell replication dose response in Conolly et al. (2003) is the large value of α_{max} (the cell replication rate corresponding to the upper end of the flux range at 15 ppm exposure) in the upward extrapolation from the empirically determined α_N(flux) (see Figure B-16 and surrounding text). The optimal value of α_{max} was found by Conolly et al. (2003) to be 0.0435 hour^{-1}. As noted by the authors, an argument in support of this value is that it corresponds to the inverse of the fastest cell cycle times found in the literature. Because the model treats the induced replication rates as being time invariant, this means that cells in the high-flux region(s) divide at the highest cell turnover rate ever observed throughout most of an animal's life. This does not seem to be biologically plausible (Subramaniam et al., 2008).

Our analysis found that a 20% increase or decrease in the estimated value for α_{max} degraded the fit to the tumor incidence data considerably. Because of the interplay among the parameters estimated by optimization, this sensitivity of the model to α_{max} indicates that it is necessary to examine if other plausible values of α_{max} are also indicated by the data and to what extent low dose estimates of risk are influenced by the uncertainty in its value. The need for such an analysis is also indicated by Figure B-18. The value of α_{max} ($\log_{10}\alpha_{max} = -1.37$) in Conolly et al. (2003) is roughly an order of magnitude greater than the values of α_N(flux) at the highest flux levels in this figure. If the data pooled over all sites and times are to be used for α_N(flux), then, based solely on the trend in α_N(flux) in Figure B-18, it appears unlikely that α_N(flux) could increase up to this value of α_{max}. Visually, these empirically derived data collectively suggest that α_N versus flux could be leveling off rather than increasing 10-fold. Therefore, as an alternative to the approach taken in Conolly et al. (2003) of estimating α_{max} via likelihood optimization against the tumor data, regressions of the empirical cell replication data in Figure B-18 were used to extrapolate α_N(flux) outside the range of observation (recognizing the uncertainty and model dependence that still results from extrapolating well outside the range of observed data).

In fitting dose-response curves to the cell replication data, a functional form was used that was flexible to allow a variety of monotonic and nonmonotonic shapes, with a parameter that determined the asymptotic behavior of the dose-response function. This allowed the extrapolation of α_N(flux) to higher flux levels by only relying on the empirical cell replication data. Then, there is no need for an adjustable parameter to be estimated by fitting to the tumor data. However, the plausible asymptotes obtained in this manner spanned a large range. In one case below, the asymptote suggested by the fit to the empirical cell replication data was judged to be abnormally high. In this case, the α_N versus flux curve was followed until the biological maximum of α_{max} [as given in Conolly et al. (2003)] was reached.

In three of the six regression models below, the data were restricted to the earliest exposure time (13 weeks) in Monticello et al. (1996) for which the cell proliferation rate (α_N) could be calculated. The interest in using only the 13-week exposure time also arises from observations (Monticello et al., 1996; 1991) that at later times there were more frequent and severe histologic changes, which may have altered formaldehyde uptake and cell proliferation response.

Consequently, given that the data in Monticello et al. (1991) for times earlier than 13 weeks could not be used as explained in the section in B.2.2 on "uncertainty due to combining pulse and continuous labeled data", the 13-week responses might better represent proliferation rates for use in a two-stage model of the cancer process than the rest of the Monticello et al. (1996) data.

Second, the LI data showed considerable variation among nasal sites, which may be related to the variation in tumor response among sites. Because the cell replication dose-response curves used in the cancer model represent all of the sites, it was attempted to include this variation by weighting the regression by the relative cell populations at risk at each of the sites. This was carried out for some of the models as stated below.

Finally, in one of the regression models, derived from fitting to all of the Monticello et al. (1996) ULLI data, time-dependence of α_N was considered by using weeks of exposure as a covariate. In this model, time was a regression (continuous) predictor, not a class variable, and its coefficient represents the change in $\log_{10} \alpha_N$ per week of exposure.

The following regression models for α_N versus flux, denoted in the equations below as N1–N6 and shown in Figures B-21 to B-26, as well as the hockey-stick and J-shaped curves used by Conolly et al. (2003), shown in Figure B-16, were next used as inputs to the clonal growth model for cancer:

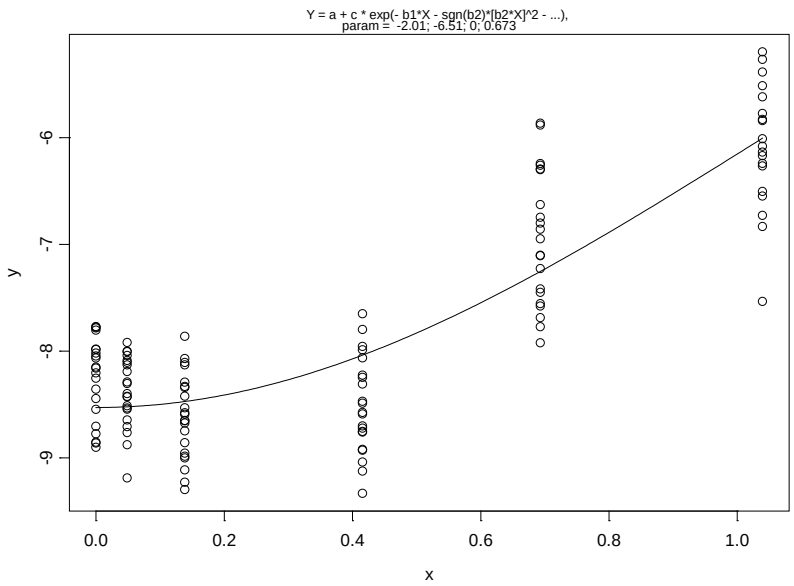


Figure B-21. Various dose-response models of normal cell replication rate; N1.

Note: See text for definitions of N1–N6. N1: Quadratic; monotone increasing in flux, derived from fit to all of the Monticello et al. (1996) ULLI data.

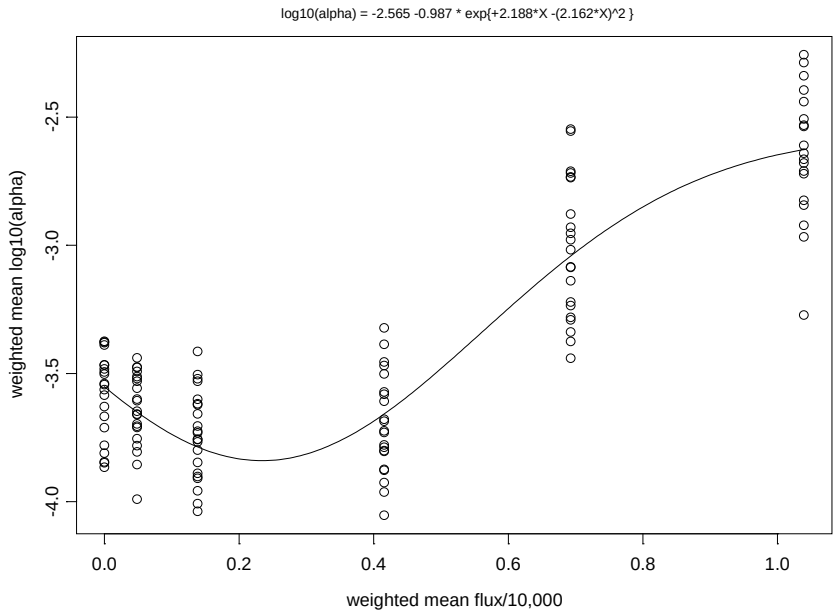


Figure B-22. Various dose-response models of normal cell replication rate; N2.

Note: See text for definitions of N1–N6. N2: Linear-quadratic; decreasing in flux for small values of flux, derived from fit to all of the Monticello et al. (1996) ULLI data.

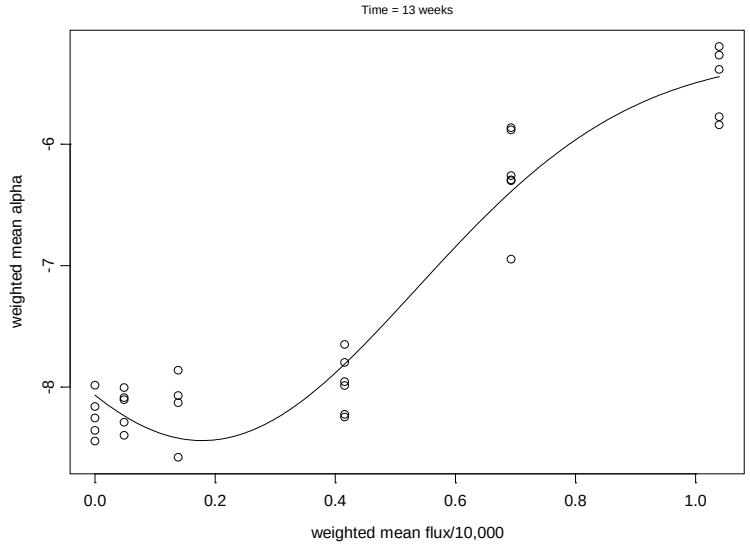


Figure B-23. Various dose-response models of normal cell replication rate; N3.

Note: See text for definitions of N1–N6. N3: Linear-quadratic; decreasing in flux for small values of flux, derived from fit to the 13-week Monticello et al. (1996) ULLI data, using average flux over all sites for a given ppm exposure and weighting regression by estimates of the numbers of cells at each of five sites.

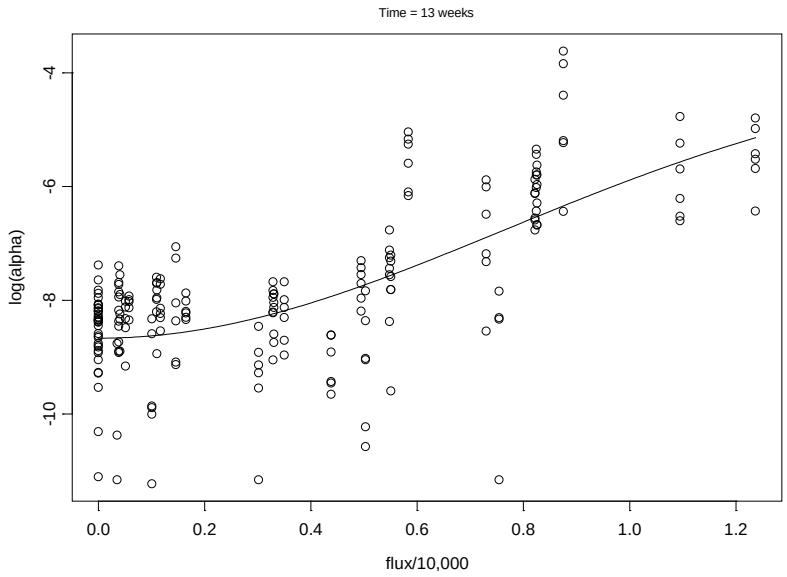


Figure B-24. Various dose-response models of normal cell replication rate; N4.

Note: See text for definitions of N1–N6. N4: Quadratic; monotone increasing in flux, derived from unweighted fit to 13-week Monticello et al. (1996) ULLI data.

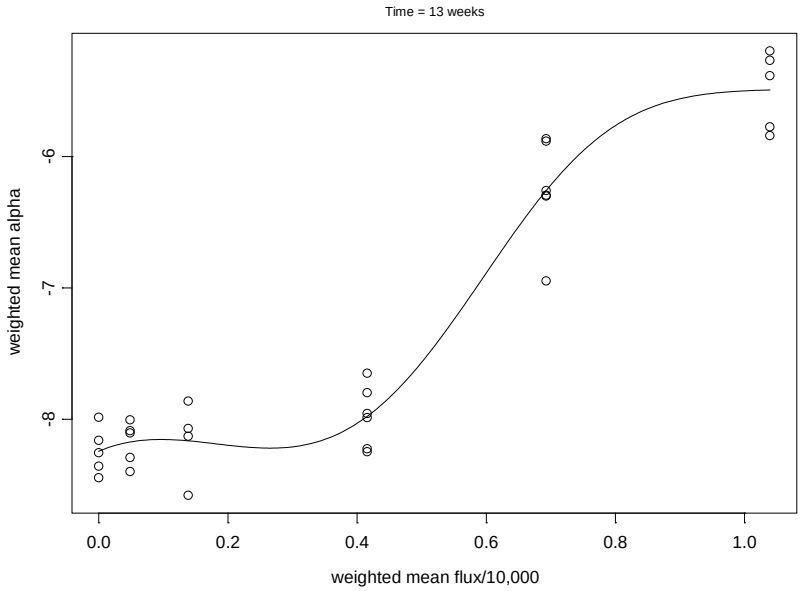


Figure B-25. Various dose-response models of normal cell replication rate; N5.

Note: See text for definitions of N1–N6. N5: Linear-quadratic-cubic; initially increasing slightly with increasing flux, then decreasing slightly, and finally increasing, derived from fit to 13-week Monticello et

al. (1996) ULLI data, using average flux over all sites for a given ppm exposure and weighting regression by estimates of the numbers of cells at each of five sites.

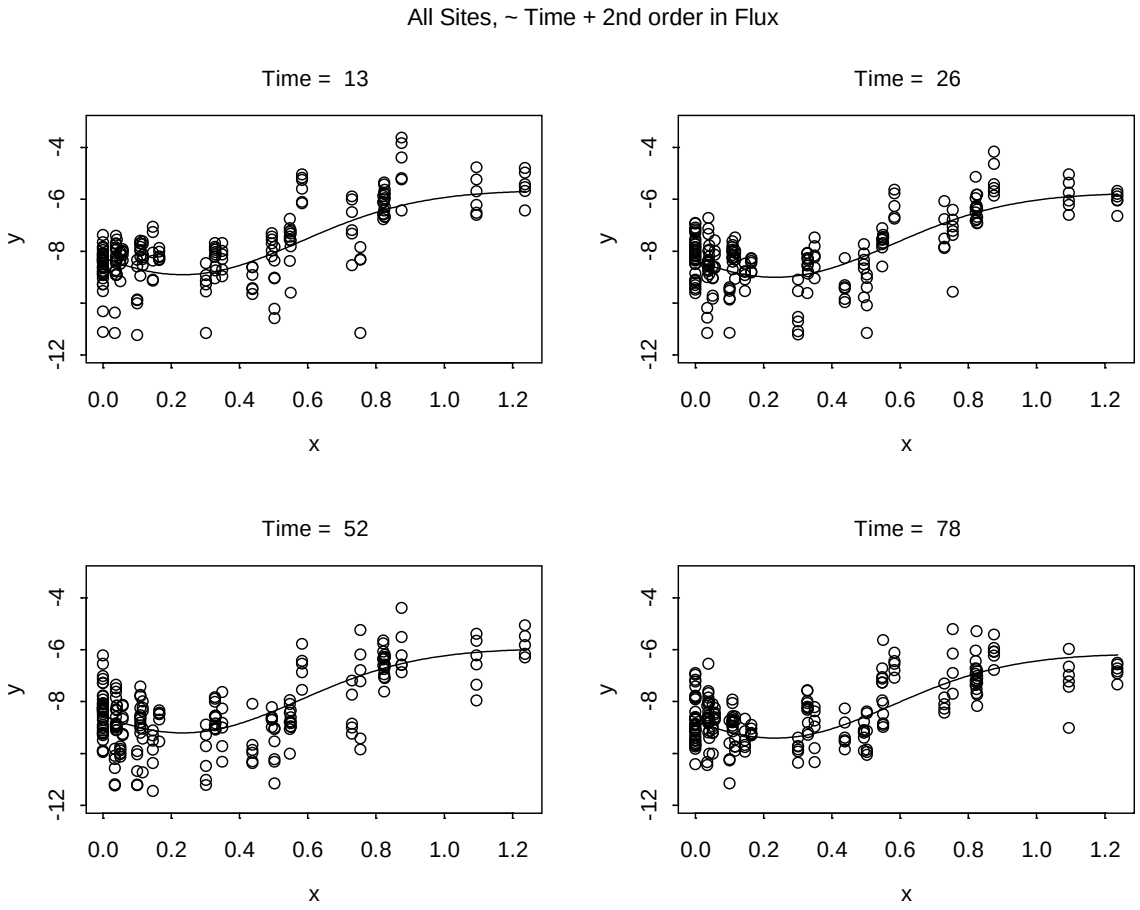


Figure B-26. Various dose-response models of normal cell replication rate; N6.

Note: See text for definitions of N1–N6. N6: Linear-quadratic-cubic; initially increasing slightly with increasing flux, then decreasing slightly, and finally increasing, derived from fit to all Monticello et al. (1996) ULLI data, using weeks of exposure as a covariate. In this model, time was a regression (continuous) predictor, not a class variable, and its coefficient represents the decrease in $\log_{10} \alpha_N$ per week of exposure time.

N1: Quadratic; monotone increasing in flux, derived from fit to all of the Monticello et al. (1996) ULLI data.

$$\alpha_N = \text{Exp}\{-2.015 - 6.513 \times \text{Exp}[-(6.735\times10^{-4} \times \text{flux})^2]\} \quad \text{(B-17)}$$

N2: Linear-quadratic; decreasing in flux for small values of flux, derived from fit to all of the Monticello et al. (1996) ULLI data.

$$\alpha_N = \text{Exp}\{-5.906 - 2.272 \times \text{Exp}[2.188\times10^{-4} \times \text{flux} - (2.162\times10^{-4} \times \text{flux})^2]\} \quad \text{(B-18)}$$

N3: Linear-quadratic; decreasing in flux for small values of flux, derived from fit to the 13-week Monticello et al. (1996) ULLI data, using average flux over all sites for a given ppm exposure and weighting regression by estimates of the numbers of cells at each of five sites.

$$\alpha_N = \text{Exp}\{-5.274 - 2.792 \times \text{Exp}[1.407\times10^{-4} \times \text{flux} - (1.986\times10^{-4} \times \text{flux})^2]\} \quad \text{(B-19)}$$

N4: Quadratic; monotone increasing in flux, derived from unweighted fit to 13-week Monticello et al. (1996) ULLI data.

$$\alpha_N = \text{Exp}\{-3.858 - 4.809 \times \text{Exp}[-(9.293\times10^{-5} \times \text{flux})^2]\} \quad \text{(B-20)}$$

N5: Linear-quadratic-cubic; initially increasing slightly with increasing flux, then decreasing slightly, and finally increasing, derived from fit to 13-week Monticello et al. (1996) ULLI data, using average flux over all sites for a given ppm exposure and weighting regression by estimates of the numbers of cells at each of five sites.

$$\alpha_N = \text{Exp}\{-5.488 - 2.755 \times \text{Exp}[-7.808\times10^{-5} \times \text{flux} + (2.349\times10^{-4} \times \text{flux})^2 - (2.166\times10^{-4} \times \text{flux})^3]\} \quad \text{(B-21)}$$

N6: Linear-quadratic-cubic; initially increasing slightly with increasing flux, then decreasing slightly, and finally increasing, derived from fit to all Monticello et al. (1996) ULLI data, using weeks of exposure as a covariate. In this model, time was a regression (continuous) predictor, not a class variable, and its coefficient represents the decrease in $\log_{10} \alpha_N$ per week of exposure time.

$$\alpha_N = \text{Exp}\{7.785\times10^{-3} \times (\text{weeks}) - 5.722 - 2.501 \times \text{Exp}[1.103\times10^{-4} \times \text{flux} - (7.223\times10^{-5} \times \text{flux})^2 - (1.575\times10^{-4} \times \text{flux})^3]\} \quad \text{(B-22)}$$

Uncertainty in model specification of kinetics of initiated cells

Biological implications of assumptions in Conolly et al. (2003)

The results of a two-stage MVK model are extremely sensitive to the values for initiated cell division (α_I) and death (β_I) rates, particularly in the case of a sharply rising dose-response curve as observed of formaldehyde. The pool of cells used for obtaining the available LI data (Monticello et al., 1996; 1991) consists of largely normal cells with perhaps increasing numbers of initiated cells at higher exposure concentrations. As such there is no way of inferring the division rates of initiated cells in the nasal epithelium, either spontaneous (baseline) or induced by exposure to formaldehyde, from the available empirical data. Conolly et al. (2003) considered α_I(flux) as a function of α_N(flux) as given by equation B-13. As shown in Figure B-16, α_I is estimated in Conolly et al. (2003) to be very similar to α_N, and a J- or hockey-shaped dose-response curve for α_N(flux) necessarily results in a J or hockey shape for α_I(flux).

The J shape for the TWA α_N(flux) in Conolly et al. (2003) could plausibly be explained, as suggested by the examples in Conolly and Lutz (2004), by a mathematical superposition of dose-response curves describing the effects of the inhibition of cell replication by the formation of DPXs (Heck and Casanova, 1999) and cytotoxicity-induced regenerative replication (Conolly, 2002). However, as explained earlier, there is considerable uncertainty and variability, both qualitative and quantitative, in the interpretation of the LI data and in the derivation of *normal* cell replication rates from the ULLI data. While the time-weighted averaged (TWA) values of ULLI indicate a J-shaped dose response for some sites, this is not consistently the case for all exposure times and sites. It is not clear why mechanisms that might explain a J-shaped or hockey-stick dose response for normal cell replication should be expected to prevail also for initiated cells.

The next critical assumption in Conolly et al. (2003) was that made for β_I (the death rate of initiated cells), namely, β_I(flux) = α_N(flux) (equation B-14). No biological justification for this assumed relationship was provided by the authors.

There are no data to evaluate the strength of these assumptions, so Subramaniam et al. (2008) studied the plausibility of various inferences that arise as a result of these assumptions. These inferences are briefly listed here.

- For flux <27,975 pmol/mm^2-hour, $\alpha_I > \alpha_N$ (see Figure B-16). Qualitatively, this concept of a growth advantage is in line with data on epithelial and other tissue types with or without exposure to specific chemicals.

- For higher flux levels in Figure B-16, the model indicates $\alpha_I < \alpha_N$. There are no data to shed further light on this inference.

- At these higher flux levels, initiated cells in the model die at a faster rate than they divide, indicating the extinction of initiated cell clones in regions subject to these flux levels. There are no data indicating formaldehyde to have this effect.

In evaluating these inferences, Subramaniam et al. (2008) point to various data that indicate that initiated cells represent distinctly different cell populations from that of normal cells with regard to proliferation response (Ceder et al., 2007b; Bull, 2000; Schulte-Hermann et al., 1997;

Coste et al., 1996; Dragan et al., 1995), have excess capacity to clear formaldehyde and, in general, are considerably more resistant to cytotoxicity, and may already have altered cell cycle control. The resistance to toxicity is manifested variably as decreased ability of the toxicant to induce cell death or to inhibit cell proliferation compared to corresponding effects in normal cells. Therefore, the influence of formaldehyde on apoptosis likely differs between normal and initiated cells.

As concluded in Subramaniam et al. (2008), taken together, there is much data to suggest that inferring $\alpha_I < \alpha_N$ at cytotoxic formaldehyde flux levels is problematic and that death rates of initiated cells are likely to be very different from those of normal cells.

In the absence of data to indicate that equations B-13 and B-14 are biologically reasonable approaches to link the kinetics of initiated cells with those of normal cells, alternate model structures other than those represented by these relationships considered by Conolly et al. (2003) were explored, given that the two-stage model is extremely sensitive to α_I and β_I. Only alternate model structures that provided a good fit to the time-to-tumor data were considered.

Plausible alternative assumptions for αI and βI

Therefore, in the additional sensitivity analysis presented here:

a) initiated cell kinetics are considered to be independent of normal cells, and
b) initiated cell replication dose response cannot take a J shape; this is motivated by the consideration that lower-than-baseline turnover rate represents an increased amount of DNA repair taking place, which may not be consistent with impaired DNA repair in initiated cells.

Thus, two alternatives were considered to equation B-13 for α_I(flux):

I1: $$\alpha_I = \gamma_1 \times [1 + \exp(\gamma_2/\gamma_3)]/\{1 + \exp[-(\text{flux} - \gamma_2)/\gamma_3]\} \quad \text{(B-23)}$$

I2: $$\alpha_I = \max[\alpha_I(\text{I1}), \alpha_{NBasal}] \quad \text{(B-24)}$$

Here γ_1, γ_2, and γ_3 are parameters estimated by fitting the cancer model to the rat bioassay data. In equation B-23, α_I increases monotonically with flux from a background level of γ_1 asymptotically up to a maximum value of $\gamma_1 \times [1 + Exp(\gamma_2/\gamma_3)]$. The choice of this functional form in was considered in order to be parsimonious while at the same time allowing for a flexible shape to the dose-response curve. The sigmoidal curve allows for the possibility of a slow rise in the curve at low dose and an asymptote.

Equation B-24 is a modification of equation B-23 that restricts the rate of division of initiated cells to be at least as large as the spontaneous division rate of unexposed normal cells. There is evidence to suggest (e.g., in the case of liver foci) that initiated cells have a growth advantage over normal cells, with or without exposure to specific chemicals (Ceder et al., 2007a; Grasl-Kraupp et al., 2000; Schulte-Hermann et al., 1999; Coste et al., 1996; Dragan et al., 1995).

In addition, in most runs, an upper bound (α_{high}) is selected for both α_N and α_I. This value is assumed to represent the largest biologically plausible rate of cell division. Following Conolly et al. (2003), in most cases α_{high} is set equal to 0.045 hours^{-1}. If a value of α_I or α_N computed using one of the above formulas exceeded α_{high}, the value of α_{high} was used in the computation rather than the value obtained by using the formula.

As noted above, Conolly et al. (2003) set the rate of death for intermediate cells, β_I, equal to the division rate of normal cells, $\beta_I = \alpha_N$. On the other hand, apoptotic rates and cell proliferation rates are thought to be coupled (Schulte-Hermann et al., 1999; Moolgavkar, 1994), so that death rates of initiated cells would rise concomitantly with an increase in their division rates (Grasl-Kraupp et al., 2000; Schulte-Hermann et al., 1999). Therefore, as an alternative to the Conolly et al. (2003) formulation, it is assumed that the death rate of intermediate cells is proportional to the division rate of intermediate cells.

$$\beta_I = K_\beta \times \alpha_I \qquad \text{(B-25)}$$

where the constant of proportionality, κ_β, is an additional parameter to be estimated by optimization against the tumor incidence data. Such an assumption has also been made by other authors (Luebeck et al., 2000; Luebeck et al., 1995; Moolgavkar et al., 1993).

Results of sensitivity analyses on αN, αI, and βI

The number of models that might be constructed if all the possibilities listed above for α_N, α_I, *and* β_I are to be tried in a systematic manner clearly become exponential and daunting. (Optimally, it would have been desirable to elucidate the role of a specific modification while keeping others unchanged to determine risk.) Therefore, in order to carry out a viable sensitivity analysis while at the same time examining the plausible range of risks resulting from variations in parameters and model structures, various uncertainties were combined in any given simulation. By using the constraints described above (equations B-17 through B-25) for α_I, β_I, and α_N, 19 models were obtained that provided similarly good fits to the time-to-tumor data (which in some cases contained only five dose groups).

However, for many of these models, the optimal α_I(flux) displayed a threshold in flux even when the model used for α_N(flux) was a monotonic increasing curve without a threshold (i.e., model N4 for α_N in Figure B-24). Indeed, if a thresholded dose-response curve was plausible for α_I based on arguments of cytotoxicity, then a threshold is all the more plausible for α_N, and such models are removed from consideration.

Secondly, the basal value of α_I was required to be at least as large as the basal value of α_N. Another constraint was placed on the baseline initiated cell replication rate. In the absence of formaldehyde exposure, α_I was not allowed to be greater than two or four times α_N, even if such

models described the tumor data, including the control data, very well. There are some data that suggest that baseline initiated cells have a small growth advantage over normal cells, so a huge advantage was thought to be biologically less plausible.

Finally, because most of the SCCs in the rat bioassays occurred in rats exposed to the highest formaldehyde concentration (15 ppm), the data from this exposure level have a big impact on the estimated model parameters. In most runs that incorporated the 15 ppm data, the model appeared, based on inspection of the KM plots, to fit the 15 ppm data quite well but to fit the lower exposure data less well. Because of the high level of necrosis occurring at 15 ppm, it is possible that the data at this exposure may not be particularly relevant to modeling the sharp upward rise in the dose response at 6 ppm. Furthermore, the principal interest is in the predictions of the model at lower levels to which human populations may be exposed. Consequently, in order to improve the fit of the model at lower exposures, some of the alternative models were constructed with the 15 ppm data omitted.

Sensitivity of risk estimates for the F344 rat

Figures B-27 and B-28 contain plots of the MLE of additional risk computed for the F344 rat at formaldehyde exposures of 0.001, 0.01, 0.1, and 1 ppm for eight models. Two log-log plots are provided. For those models for which the estimates of additional risk are all positive, the additional risks are plotted (Figure B-27), and, for those for which estimates of additional risk are negative, the negatives of additional risks are plotted (Figure B-28). Only five dose groups were considered (i.e., 15 ppm data omitted) for models 8, 5, 15, and 16. Figures 29 and 30 show the dose-response curves for α_N and α_I for these eight cases (corresponding to those in Figures B-27 and B-28 respectively). The specification and estimated values of the parameters for these models are provided in Tables B-23 and B-24. The primary results are as follows:

1) Among the models considered, negative values for additional risk can arise only in models in which the dose response for normal cells is J shaped. Thus, all of the models with negative dose responses for risk have J-shaped dose responses for normal cells. However, the converse is not necessarily true as may be noted from model 8. This model has both a positive dose response for risk and a J-shaped dose response for normal cells. In this case, the strong positive increase in response of initiated cells at low dose was sufficient to counteract the negative response of normal cells.

2) For doses below which no tumors were observed, the risk estimates predicted by the different models span a very large range. This result points to large uncertainties in model specification (how to relate the kinetics of normal and initiated cells) as well as in parameter values. As mentioned above, the analysis does not attempt to separate the influence of the different sources of uncertainty, so this range also incorporates the uncertainty arising from the use of different control data and that due to α_{max}.

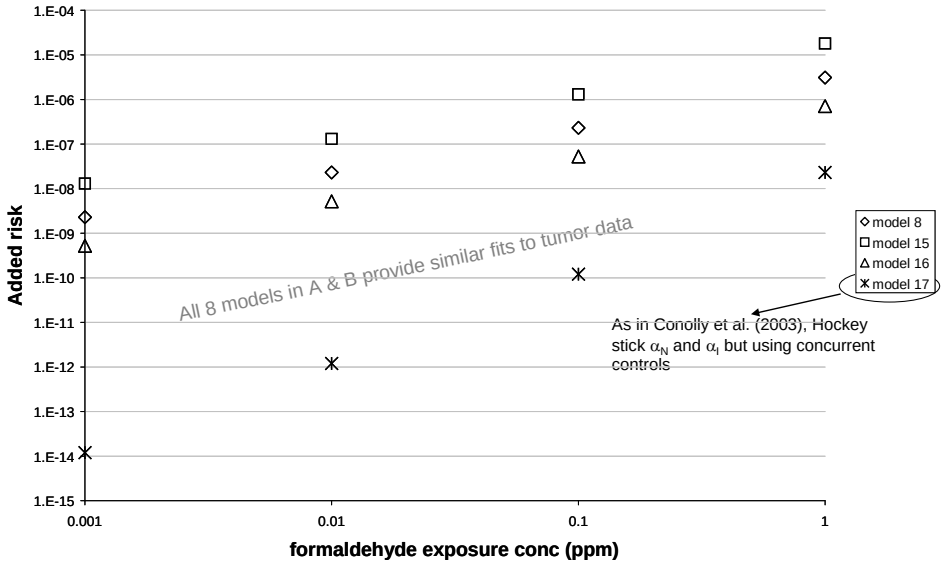


Figure B-27. BBDR models for the rat—models with positive added risk.

Note: All four models provide "similar" fits to tumor data (see text)

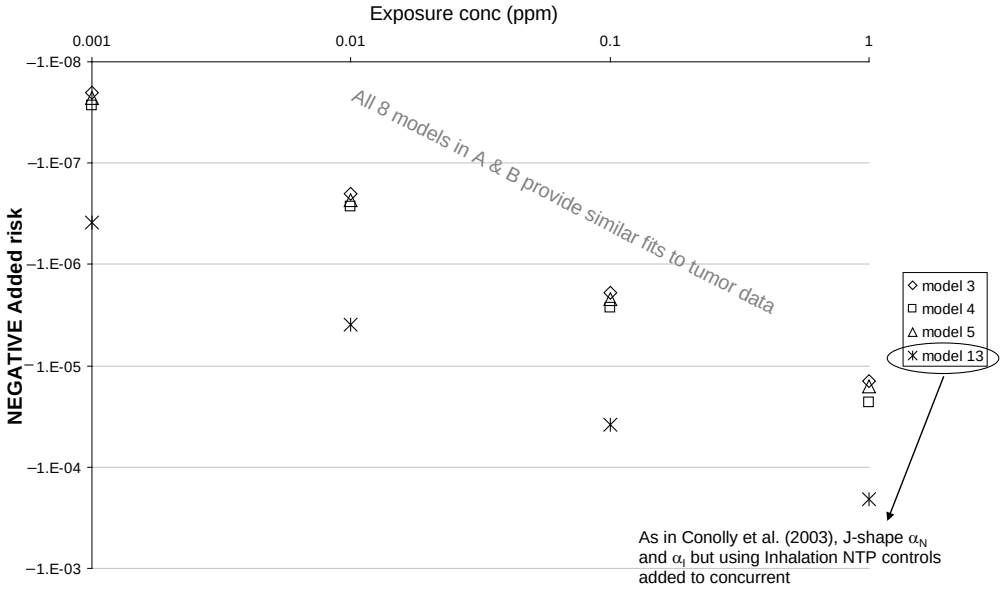


Figure B-28. BBDR rat models resulting in negative added risk.

Note: All four models provide "similar" fits to tumor data (see text).

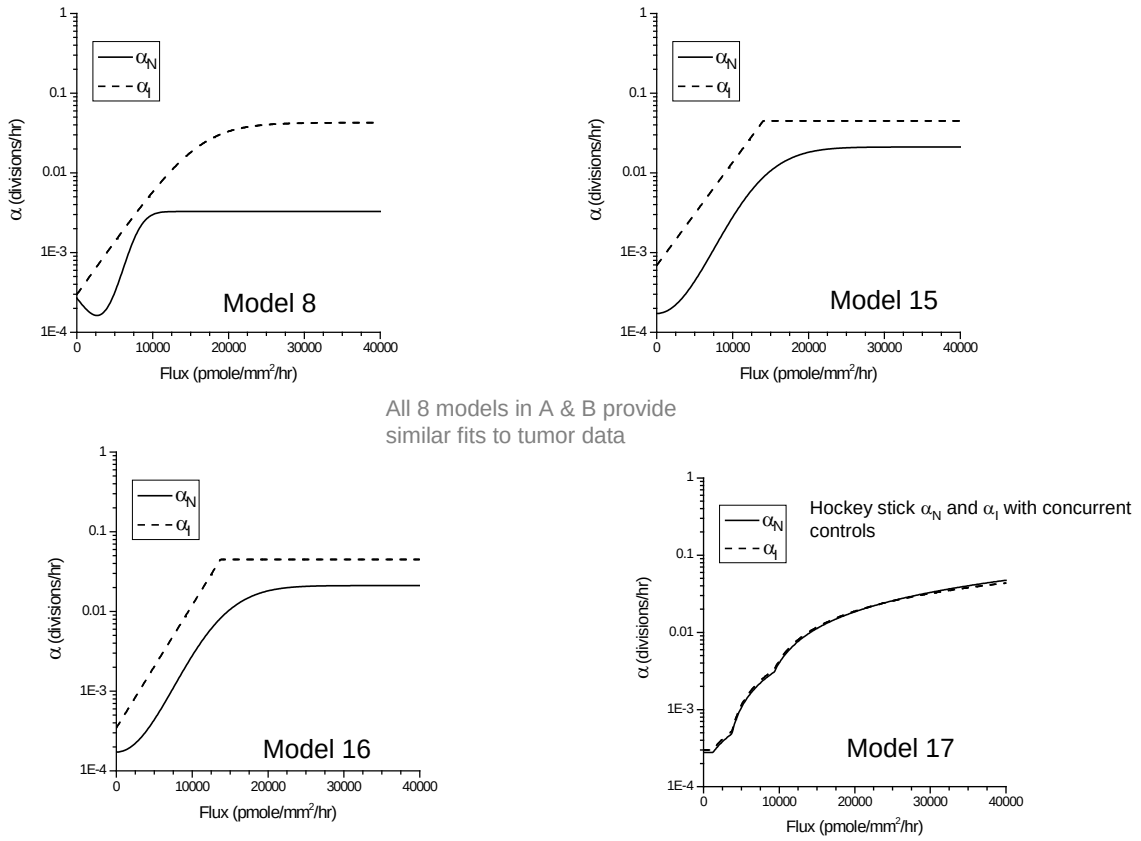


Figure B-29. Models resulting in positive added rat risk: Dose response for normal and initiated cell replication.

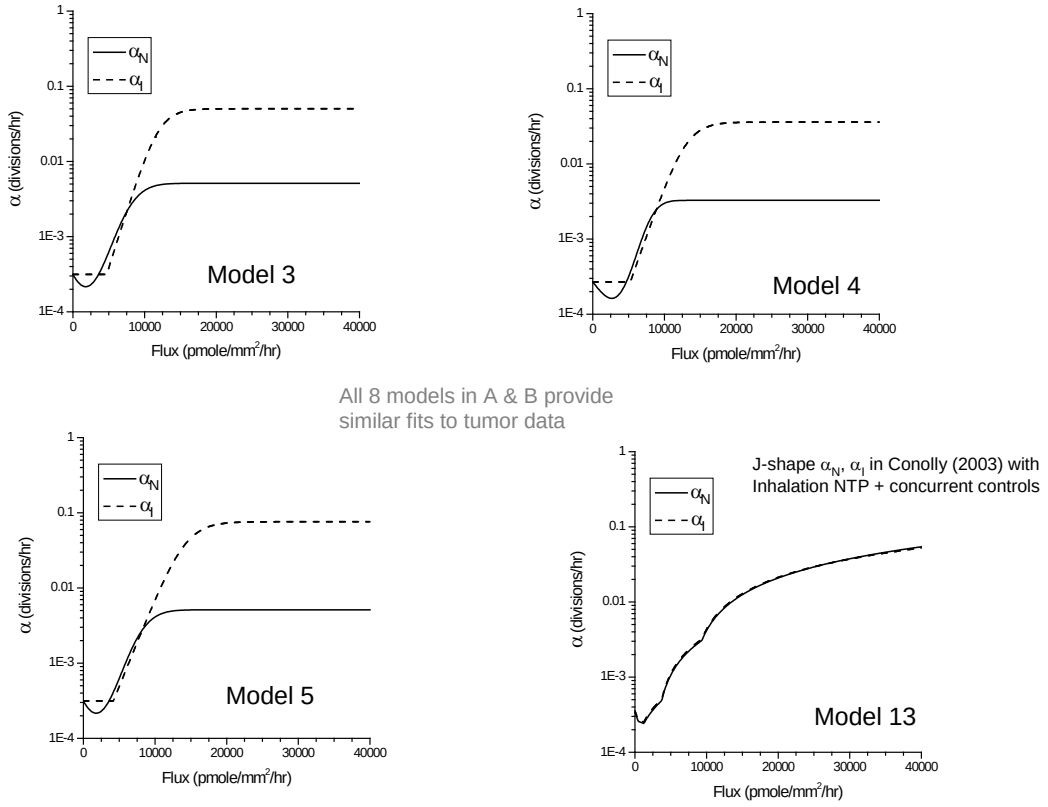


Figure B-30. Models resulting in negative added rat risk: Dose response for normal and initiated cell replication.

Table B-23. Parameter specifications and estimates for clonal growth models of nasal SCC in the F344 rat using alternative characterization of cell replication and death rates

Parameters	Model 3	Model 4	Model 5	Model 8	Model 15	Model 16
Historical controls added to concurrent	Inhalation NTP	Inhalation NTP	Inhalation NTP	Inhalation NTP	Inhalation NTP	Inhalation NTP
Number of dose groups	6	6	5	5	5	5
DPX concentration	Subramaniam et al. (2007)	Subramaniam et al. (2007)	Subramaniam et al. (2007)	Subramaniam et al. (2007)	Subramaniam et al. (2007)	Subramaniam et al. (2007)
α_N definition	N3	N6	N3	N6	N4	N4
α_I definition	I2	I2	I2	I1	I1	I1
α_{high}	--	0.045	--	0.045	0.045	0.045
β_I definition	$\beta_I = K_\beta \times \alpha_I$	$\beta_I = K_\beta \times \alpha_I$	$\beta_I = K_\beta \times \alpha_I$	$\beta_I = K_\beta \times \alpha_I$	$\beta_I = K_\beta \times \alpha_I$	$\beta_I = K_\beta \times \alpha_I$
					$\gamma_1 \leq 4\ \alpha_{NBasal}$	$\gamma_1 \leq 2\ \alpha_{NBasal}$
Log-likelihood	-1,495.34	-1,495.61	-184.02	-184.22	-182.75	-186.37
μ_{NBasal}	7.518×10^{-7}	1.664×10^{-6}	8.684×10^{-7}	9.230×10^{-7}	1.037×10^{-6}	1.662×10^{-7}

Parameters	Model 3	Model 4	Model 5	Model 8	Model 15	Model 16
KMU	3.884×10^{-7}	3.471×10^{-7}	0.0	0.0 (0.0, 2.093×10^{-6})	4.582E-6 (1.8×10^{-6},1.86×10^{-5})	0.0
KMX (*KMU*/μ_{NBasal})	0.5166	0.2086	0.0	0.0 (0.0, 4.696)	4.420 (1.53, 17.67)	0.0
D_0[§]	214.3	199.7	261.8	254.2	423.2	245.1
D_{0F}[§]	75.26	79.81	119.7	101.1	100.8	98.83
γ_1	1.164×10^{-5}	1.006×10^{-5}	3.168×10^{-5}	2.967×10^{-4}	6.888×10^{-4}	3.441×10^{-4}
γ_2	1427	1,591	1,825	3,223	4,652	2,818
γ_3	11,944	13,017	14,207	15,989	54,334	37,896
K_β	0.9893	0.9848	0.9804	0.9504	1.006	0.9660

[§]See Subramaniam et al. (2007) for an explanation of the time delay constants D_0 and D_{0F}

Table B-24. Parameter specifications and estimates for clonal growth models of nasal SCC in the F344 rat using cell replication and death rates as characterized in Conolly et al. (2003)

Parameters	Model 13	Model 17
Historical controls added to concurrent	All NTP	NO historical controls
Number of dose groups	6	6
DPX concentration	Conolly et al. (2000)	Subramaniam et al. (2007)
α_N definition	J shape [TWA, Conolly et al. (2003)]	Hockey [TWA, Conolly et al. (2003)]
α_I definition	eq. B-13	eq. B-13
α_{high}	--	--
β_I definition	$\beta_I = \alpha_N$	$\beta_I = \alpha_N$
Log-likelihood	−1,692.68	−1,474.29
μ_{NBasal}	1.731×10^{-6}	0.0
KMU	0.0	1.203×10^{-6} (1.0×10^{-6},1.427×10^{-6})
KMX (*KMU*:μ_{NBasal})	0.0	Infinite (0.4097, infinite)
D_0[§]	239.5	243.13
D_{0F}[§]	66.31	68.83
multib	1.047	$1.078 \times 10^{+0}$
multic	1.510	3.347
α_{max}	5.153×10^{-2}	0.045

[§]See Subramaniam et al. (2007) for an explanation of the time delay constants D_0 and D_{0F}

1) At the 10 ppb (0.01 ppm) concentration, MLE risks range from -4.0×10^{-6} to $+1.3 \times 10^{-7}$. At this dose, models that gave only positive risks resulted in a five orders of magnitude risk range from 1.2×10^{-12} to 1.3×10^{-7}, while narrowing to a four orders of magnitude risk range from 1.2×10^{-10} to 1.3×10^{-6} at the 0.1 ppm level. This narrowing continues as exposure concentration increases, and the curves coalesce to substantially similar values at 6 ppm and above (not shown). For all these 8 models, the rat added risk at 6.0 ppm ranged from 1.8×10^{-2} to 2.1×10^{-2}.

2) There does not seem to be any systematic effect on additional risk that depends on whether the 15 ppm data are included in the analysis.

3) For all of the models except Models 13 and 17 in Figures B-27 and B-28, the additional risk varies substantially linearly with exposure at low exposures between 0.001 and 1.0 ppm (departing only to a small extent from linearity between 0.1 and 1.0 ppm). Models 13 and 17 show a quadratic dependence; these models employ the TWA J-shaped and hockey stick dose-response curves for α_N used in Conolly et al. (2003) and the same equations used by those authors to relate α_I and β_I to α_N (equations B-13 and B-14). However, the control data in Model 17 was different from those used by Conolly et al.; while all NTP controls were added to the concurrent controls in Model 13, only concurrent controls were used in Model 17.

The various model choices presented in Figures B-29 and B-30 all provided equally good fits to the time-to-tumor data although within the context of a significant qualification. It was not possible to simply use the maximized log-likelihood values as a means of comparing the goodness of fit to the tumor incidence data across all these model choices. This is because many of the model choices differed in the number of doses or in the number of control animals that were used, so the fits were compared across such models only visually.

Wherever results from the BBDR modeling are discussed, values of added risk, as opposed to extra risk, are reported. This is purely for convenience in interpretation. Because of the low background incidence, these values are only negligibly different from the corresponding extra risk estimate. The final risk (or unit risk) estimates provided in this document are based on extra risk estimates.

Confidence bounds: model uncertainty versus statistical uncertainty

For Models 15 and 17 in Figures B-29 and B-30, 90% CIs for additional risk were calculated by using the profile-likelihood method. Table B-25 compares the lower and upper confidence bounds for these models for 0.001 ppm, 0.1 ppm (doses well below the range where tumors were observed), and 6 ppm (the lowest dose where tumors were observed) with the MLE risk estimates at these doses. In both cases, these intervals were quite narrow compared with the differences in risk predicted by the different models. This suggests that model uncertainty is of more consequence in the formaldehyde animal model than is statistical uncertainty. We also estimated confidence bounds using the bootstrap method for select models and determined that these estimates were in agreement with the bounds calculated using the profile-likelihood method.

These results are not presented here. We return to the calculation of confidence limits when determining points of departure (PODs).

Table B-25. Comparison of statistical confidence bounds on added risk for two models

Dose (ppm)	Model	Lower bound	MLE	Upper bound
0.001	Model 15	4.4×10^{-9}	1.3×10^{-8}	1.6×10^{-8}
	Model 17	1.2×10^{-14}	1.2×10^{-14}	1.3×10^{-14}
0.1	Model 15	4.5×10^{-7}	1.3×10^{-6}	1.7×10^{-6}
	Model 17	1.2×10^{-10}	1.2×10^{-10}	1.3×10^{-10}
6	Model 15	1.8×10^{-2}	2.1×10^{-2}	2.3×10^{-2}
	Model 17	1.3×10^{-2}	1.8×10^{-2}	3.0×10^{-2}

In conclusion, it is demonstrated that the different formaldehyde clonal growth models can fit the data about equally well and still produce considerable variation in additional risk and biological inferences at low exposures.

Statistical Methods Used in Evaluation

Parameters of the alternate models shown here were estimated by maximizing the likelihood function defined by the data (Cox and Hinkley, 1974). Such estimates are referred to as maximum likelihood estimates (MLEs). Statistical confidence bounds were computed by using the profile-likelihood method (Crump, 2002; Cox and Oakes, 1984; Cox and Hinkley, 1974). In this approach, an asymptotic $100(1 - \alpha)\%$ upper (lower) statistical confidence bound for a parameter, β, in the animal cancer model is calculated as the largest (smallest) value of β that satisfies

$$2[L_{max} - L^*(\beta)] = x_{1-2\alpha} \quad \text{(B-26)}$$

where L indicates the likelihood of the rat bioassay data, L_{max} is its maximum value, $L^*(\beta)$ is, for a fixed value of β, the maximum value of the log-likelihood with respect to all of the remaining parameters, and $x_{1-2\alpha}$ is the $100(1-2\alpha)$ percentage point of the chi-square distribution with one degree of freedom. The required bound for a parameter, β, was determined via a numerical search for a value of β that satisfies this equation.

The additional risk is defined as the probability of an animal dying from an SCC by the age of 790 days, in the absence of other competing risks of death, while exposed throughout life to a prescribed constant air concentration of formaldehyde, minus the corresponding probability in an animal not exposed to formaldehyde. The MLE of additional risk is the additional risk computed using MLEs of the model parameters.

The method described above for computing profile-likelihood confidence bounds cannot be used with additional risk because additional risk is not a parameter in the cancer model. Instead, an asymptotic $100(1 - \alpha)\%$ upper (lower) statistical confidence bound for additional risk was

computed by finding the parameter values that presented the largest (smallest) value of additional risk, subject to the inequality

$$2[L_{max} - L] \leq x_{1-2\alpha} \quad \text{(B-27)}$$

being satisfied, with the resulting value of additional risk being the required bound. This procedure was implemented through use of penalty functions (Smith and Coit, 1995). For example, the profile upper bound on additional risk was computed by maximizing the "penalized added risk," defined as (*additional risk – penalty*), where

$$\text{penalty} = W \times \{[(L_{max} - L) - x_{1-2\alpha}/2]^{+}\}^2 \quad \text{(B-28)}$$

and $[\ ]^{+}$ equals the quantity in the brackets whenever it is positive and zero otherwise. The multiplicative weight, *W*, was selected by trial and error so that the final solution satisfied the following equation sufficiently well.

$$2(L_{max} - L) = x_{1-2\alpha} \quad \text{(B-29)}$$

The computer code was written in Microsoft Excel 2002 SP3 Visual Basic. Either the regular Excel Solver or the Frontline Systems Premium Solver was used to make the required function optimizations. Computation of confidence bounds was highly computationally intensive, and, consequently, confidence bounds were computed only for selected parameters in selected runs. For select cases, the bootstrap method was also used to calculate confidence bounds in order to confirm their accuracy. Values so calculated were found to be in agreement with those calculated by using the likelihood method.

Sensitivity Analysis of Conolly et al. (2004) Human Extrapolation Model

Uncertainties in the Human Extrapolation Model

Subsequent to the BBDR model for modeling rat cancer, Conolly et al. (2004) developed a corresponding model for humans for the purpose of extrapolating the risk estimated by the rat model to humans. Also, rather than considering only nasal tumors, it is used to predict the risk of all human respiratory tumors. The human model for formaldehyde carcinogenicity (Conolly et al., 2004) is conceptually very similar to the rat model but is not based on any data on human exposure to formaldehyde. Unlike the sensitivity analysis of the rat modeling where a number of issues were examined, a much more restricted analysis will be presented here for the sake of brevity. A more extensive analysis was carried out initially that carried forward several of the rat models in B.2.2 to the human, and the lessons learned from those exercises are in agreement with the more restricted presentation that follows. Table B-26 lists the major uncertainties and assumptions in the human extrapolation model in Conolly et al. (2004).

Table B-26. Summary of evaluation of major assumptions and results in Conolly et al. (2004)

Assumptionsa	Rationale in Conolly (2004, 93075) or CIIT (1999)	EPA evaluation	Further elaboration
Cell division rates derived from rat labeling data were assumed applicable to human (except for assuming different fraction of cells with replicative potential).	There are no equivalent LI data for human or guidance for extrapolating cell division rate across species.	Enzymatic metabolism plays a role in mitosis. Therefore, we expect interspecies difference in cell division rate. Basal cell division rates in humans are expected to be much more variable than in laboratory animals.	Subramaniam et al. (2008)
Parameters for enzymatic metabolism of formaldehyde in human PBPK model for DPX concentrations: K_m varies by order of magnitude between rat and monkey but is same for monkey and human. V_{max}:K_m is similar for rat and monkey but 6-fold lower for human.	See "PBPK model for Human DPX…"	See "PBPK model for Human DPX…"	"PBPK model for Human DPX…"; Conolly et al. (2000); Subramaniam et al. (2008); Klein et al. (2011)
Anatomically realistic representation of nasal passages.	Reduces uncertainty (over default calculation carried out by averaging dose over entire nasal surface).	Computer representation pertains to that of one individual (white male adult). There is considerable interindividual variability in nasal anatomy. Susceptible individuals are even more variable.	Kimbell et al. (2001b; 2001);Subramaniam et al. (2008; 1998)
KMU:μ_{Nbasal} is species invariant (used to estimate human).	Human cells are more difficult to transform than rodent, both spontaneously and by exposure to formaldehyde.	μ_{Nbasal} is 0 when concurrent controls or inhalation NTP controls in time frame of concurrent bioassays are used. Leads to infinitely large KMU for human.	Subramaniam et al. (2007); Crump et al. (2009); (Crump et al., 2008).

Assumptionsa	Rationale in Conolly (2004, 93075) or CIIT (1999)	EPA evaluation	Further elaboration
Conservative assumptions were made. Results are conservative in the face of model uncertainties.	1) Hockey-stick dose response for α_N was included even though TWA indicated J shape. 2) Overall respiratory tract cancer incidence data for human baseline rates were used. 3) Risk was evaluated at statistical upper bound of the proportionality parameter relating DPXs to the probability of mutation.	Results in Conolly et al. (2004) are not conservative in the face of model uncertainties: (a) human risk estimates are very sensitive to use of historical controls in the analysis of the animal bioassay, (b) human risk estimates are unboundedly large when concurrent controls are used in rat model, and (c) minor perturbations in model assumptions regarding division and death rates of initiated cells lead to upper bound risks that were more than 1,000-fold greater than the highest estimates in Conolly et al. (2004).	Conolly et al. (2004); Subramaniam et al. (2007); Crump et al. (2009); (Crump et al., 2008).

[a]Assumptions in this table are in addition to those listed for the BBDR model for the F344 rat.

Uncertainties in the PBPK Model for Human DPX Concentrations

Conolly et al. (2000) constructed a PBPK model for the rhesus monkey along similar lines as for the F344 rat, and used the rat and rhesus monkey parameter estimates to develop a model for human DPX concentrations. In the rhesus monkey model, they maintained the same values of k_b, k_{loss}, and k_f as in the rat model but optimized the values of Vmax and Km against the rhesus monkey data from Casanova et al. (1994). The resulting human PBPK model used formaldehyde flux estimates predicted by an anatomically realistic CFD modeling of the nasal passages; except for the anatomic reconstruction, there were no other human data used to inform the PBPK model.

For the human, the model used the value of K_m estimated by the rhesus monkey model and the epithelial thickness averaged over three regions of the rhesus monkey nose. The maximum rate of metabolism, Vmax, which was estimated independently for the rat and rhesus monkey by fitting to the DPX data available for these species, was then extrapolated to the human by assuming a power law scaling with body weight (BW) (i.e., $Vmax = a \times BW^b$), and the coefficient "a" and exponent "b" were derived from the independently estimated values of $(Vmax)_{RAT}$ and $(Vmax)_{MONKEY}$. Table B-27 gives the values of Vmax and Km in the Conolly et al. (2000) extrapolation.

Table B-27. Extrapolation of parameters for enzymatic metabolism to the human in Conolly et al. (2000)

Parameter	F344 rat	Rhesus monkey	Human
Vmax (pmol/min-mm^3)	1,008.0	91.0	15.7
Km (pmol/mm^3)	70.8	6.69	6.69

Source: Conolly et al. (2000).

In general, laws for allometric scaling across species, such as how enzymatic metabolic rates vary across organisms, are derived as empirical regression relationships based on data from multiple species and usually multiple sources of data points. For example, West and Brown (2005) demonstrate that metabolic rates scale with mass$^{3/4}$ using data from organisms ranging over 27 orders of magnitude in mass (intracellular up to the largest organisms). In Conolly et al. (2000), the power-law relationship is derived using two data points (F344 rat and rhesus monkey for a single chemical) with log BW as *x*-axis and Vmax on *y*-axis. Because such a regression does not have the power to delineate the curvature in the scaling function, the empirical strength of the allometric relationship derived in Conolly et al. (2000) is extremely weak for use in extrapolating from the rat to the human on the basis of body-weight. Furthermore, as noted earlier, V_{max} is highly correlated to K_m, the value of K_m appears to vary substantially between the rat and monkey, and as indicated by the large standard error using multiple methods in Klein et al. (2011), its estimation is fairly uncertain. These observations make the scaling relationship in Conolly et al. (2000) more problematic.

The following observations point to the uncertainty in the values of the parameters Vmax and K_m in the Conolly et al. (2000) models for predicting DPXs. First, Km varies by an order of magnitude across the rat and monkey models and considered invariant between the monkey and human models (Conolly et al., 2000). Second, the values in Conolly et al. (2000) for Vmax/Km, the low-dose limit of the rate of enzymatic metabolism, is roughly similar between the rat and monkey but lower by a factor of six in the human.

Another factor that can substantially influence the above extrapolation of DPXs in the human is that Conolly et al. (2000) assumed the tissue to be a well-mixed compartment with regard to formaldehyde interaction with DNA and used the amount of formaldehyde bound to DNA per unit volume of tissue as the DPX dose metric. Considering formaldehyde's highly reactive nature, the concentrations of formaldehyde and DPX are likely to have a sharp gradient with distance into the nasal mucosa (Georgieva et al., 2003). Cohen Hubal et al. (1997) concluded that the well-mixed assumption is inappropriate at exposure concentrations less than 4 ppm. Furthermore, given the interspecies differences in tissue thickness, there is uncertainty as to whether DPX per unit volume or DPX per unit area of nasal lining is the more appropriate dose metric to be used in the extrapolation. In particular, it may be assumed that the cells at risk for tumor formation are only those in the epithelium and that measured DPX data (in monkeys and rats) are an average over the entire tissue thickness. Because the epithelial DPXs in monkeys (and presumably humans) would then be more greatly "diluted" by lower levels of DPX formation that occur deeper into the tissue

than in rats, it could be predicted that the ratio of epithelial to measured DPXs in monkeys and humans would be much higher than the ratio in rats.

On the whole, these observations suggest that human extrapolations of DPX concentrations using the human PBPK model in Conolly et al. (2000) may be highly uncertain.

Sensitivity Analysis of Clonal Growth Model for Human Extrapolation

EPA (Crump et al. (2008)) carried out a limited sensitivity analysis of the Conolly et al. (2004) human model. This analysis was limited to evaluating the effect on the human model of the following. These evaluations have been the subject of some debate in the literature and at various conferences (Conolly et al., 2009; Crump et al., 2009).

1) The use of the alternative sets of control data for the rat bioassay data that were considered in the sensitivity analysis of the rat model in B.2.2.

2) Minor perturbations in model assumptions regarding the effect of formaldehyde on the division and death rates of initiated cells (α_I, β_I).

One (of the two) adjustable parameter in the expression for the human α_I in Conolly et al. (2004) was determined from the model fit to the rat tumor incidence data while the second parameter was determined from background rates of cancer incidence in the human. Therefore, variations considered in α_I were constrained to only those that (a) did not meaningfully degrade the fit of the model to the rat tumor incidence data, as shown in Figure B-34, and (b) were in concordance with background rates in the human.

Crump et al. (2008). also evaluated these variations with respect to their biological plausibility. The sensitivity analysis on assumed initiated cell kinetics was thought to be particularly important because there were no data to even crudely inform the kinetics of initiated cells for use in the models, even in rats, and the two-stage clonal expansion model is very sensitive to initiated cell kinetics (Gaylor and Zheng, 1996; Crump, 1994 1994, 064809).

Effect of background rates of nasal tumors in rats on human risk estimates

Crump et al. (2008) quantitatively evaluated the impact of different control groups on estimates of additional human risk as follows:

1) Concurrent controls plus all NTP controls:, the same as used by Conolly et al. (2004);

2) Concurrent controls plus controls from NTP inhalation studies;

3) Only concurrent controls;

4) Each set of control data was applied with both the J shape and hockey-stick models in Conolly et al. (2004) for α_N(flux) and α_I(flux) for a total of six analyses.

5) Uncertainties associated with α_N or α_I are not addressed. Parameters α_{max}, multfc, and KMU were estimated in exactly the same manner as in Conolly et al. (2004).

Crump et al. (2008) present the following dose-response predictions of additional risk in humans from constant lifetime exposure to various levels of formaldehyde arising from exercising the above six cases. Their plots are reproduced in Figure F-1, where the corresponding curves based on Conolly et al. (2004) are also shown for comparison.

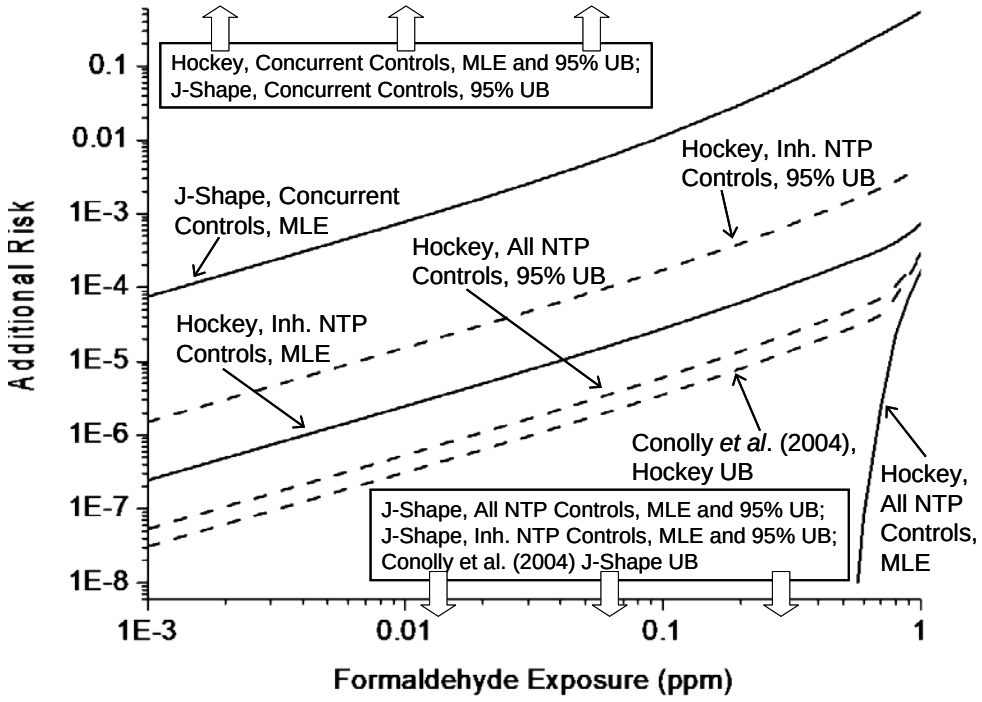


Figure B-31. Effect of choice of NTP bioassays for historical controls on human risk.

Note: Estimates of additional human risk of respiratory cancer by age 80 from lifetime exposure to formaldehyde are obtained by using different control groups of rats.

Source: Crump et al. (2008).

The lowest dotted curve in Figure B-31 represents the highest estimates of human risk developed by Conolly et al. (2004). This resulted from use of the hockey-stick model for cell division rates in conjunction with the statistical upper bound for the parameter KMU. As indicated by the downward block arrows in the figure, their corresponding estimates based on the J-shaped model were all negative for exposures below 1 ppm.

Consider next the solid curves in the figure, which show predicted MLE added risks that were positive and less than 0.5. Crump et al. (2008) next examined the added risk obtained when the MLE estimate of (KMU:μ_{basal}) in these cases is replaced by the 95% upper bound of this parameter ratio. The upper bound risk estimates in Conolly et al. (2004) were calculated in a similar manner (but using all NTP historical controls). Except for minor differences, risk estimates

corresponding to such an upper bound and using all NTP controls were very similar in the two efforts (Crump et al., 2008; Conolly et al., 2004).

Figure B-31 shows that the choice of controls to include in the rat model can make an enormous difference in estimates of additional human risk. For the J-shaped model for cell replication rate both estimates based on the MLE and those based on the 95% upper bound on $KMU{:}\mu_{basal}$ are negative for formaldehyde exposures below 1 ppm. However, when only concurrent controls are used in the model in Crump et al. (2008), the MLE from the J-shaped model is positive and is more than three orders of magnitude higher than the highest estimates obtained by Conolly et al. (2004). Using only concurrent controls, estimates based on the 95% upper bound on $KMU{:}\mu_{basal}$ are unboundedly large (block arrows at the top of the figure). For the hockey-stick shaped model for cell replication rate, when all NTP controls are used, the estimates based on the MLEs are zero for exposures less than about 0.5 ppm. If only inhalation controls are added, the MLEs are about seven times larger than the Conolly et al. (2004) upper bound estimates, and the estimates based on the 95% upper bound on $KMU{:}\mu_{basal}$ are about 50 times larger than the Conolly et al. (2004) estimates. If only concurrent controls are used, both the MLE estimates and those based on the 95% upper bound on $KMU{:}\mu_{basal}$ are unboundedly large.

Alternative assumptions regarding the rate of replication of initiated cells

For the human model, Conolly et al. (2004) made the same assumptions for relating α_I(flux) and β_I(flux) to α_N(flux) as in their rat model (Conolly et al., 2003). That is, these quantities were related by using equations B-13 and B-14. By extending the shape of these curves to humans, the authors' model brings the cytotoxic action of formaldehyde to bear strongly on the parameterization of the human model as well.

In the sensitivity analyses that follows, calculations similar to that presented in Table 2-25 of the Toxicological Review are continued over a large range of exposure concentrations. In these analyses, Crump et al. (2008) made minor modifications to the assumed division rates of initiated cells in Conolly et al. (2004), while all other aspects of the model and input data were kept unchanged. Two alternatives were considered for each of the J-shaped and hockey-stick models. Figure B-32 shows the hockey-stick model for initiated cells in rats. In the first modification to the hockey-stick model (hockey-stick Mod 1), rather than having a threshold at a flux of 1,240 pmol/m^2-hour, the division rate increases linearly with increasing flux until the graph intersects the original curve at 4,500 pmol/m^2-hour, where it then assumes the same value as in the original curve for larger values of flux. The second modification (hockey-stick Mod 2) is similar, except the modified curve intersects the original curve at a flux of 3,000 pmol/m^2-hour.

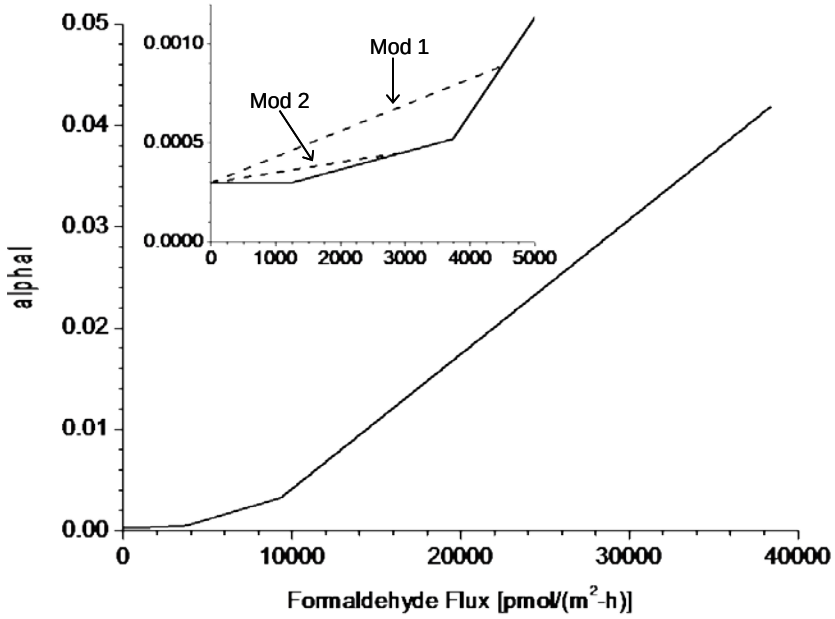


Figure B-32. Variations to the hockey-stick model for division rates of initiated cells in rats.

Source: Crump et al. (2008).

Figure B-33 shows the rat J-shaped model for initiated cells. In the first modification to this dose response (J-shaped Mod 1), rather than having a J shape, the division rate of initiated cells remains constant at the basal value until the original curve rises above the basal value and has the same value as the original curve for larger values of flux. In the second modification (J-shaped Mod 2), the J shape is retained but somewhat mitigated. In this modification, the division rate initially decreases in a linear manner similar to that of the original model but with a less negative slope until it intersects the original curve at a flux of 1,240 μm/m²-hour, where it then follows the original curve for higher values of flux.

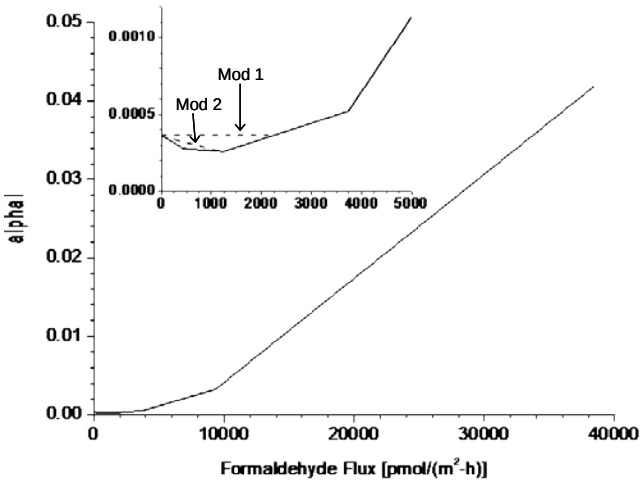


Figure B-33. Variations to the J-shaped model for division rates of initiated cells in rats.

Source: Crump et al. (2008).

Because the first constraint on the variation in α_I was in concordance with the rat time-to-tumor incidence data, Crump et al. (2008) applied each of the modified models in Figures B-32 abd B-33 to the version of the formaldehyde models in Subramaniam et al. (2007) that employed all NTP controls and the hockey-stick curve for α_N. These authors restricted their analysis to this case because their stated purpose was only a sensitivity analysis as opposed to developing alternate credible risk estimates. Figure B-34 reproduces [from Crump et al. (2008)] curves of the cumulative probability of a rat dying from a nasal SCC by a given age for bioassay exposure groups of 6, 10, and 15 ppm. For comparison purposes, the corresponding KM (nonparametric) estimates of the probability of death from a nasal tumor are also shown. Three sets of probabilities are graphed: the original unmodified one and the ones obtained by using hockey-stick Mod 1 and Mod 2. Crump et al. (2008) state that the changes in the tumor probability resulting from these modifications are so slight that the three models cannot be readily distinguished in this graph.[34] Thus, the modifications considered to the models for the division rates of initiated cells caused an inconsequential change in the fit of the model-predicted tumor incidence to the animal tumor data.

[34]The largest change in the tumor probability resulting from this modification for any dose group and any age up through 900 days was found to be less than 0.002, a change so small that it would be impossible to detect, even in the largest bioassays ever conducted. The changes in tumor probability resulting from the other modifications described earlier were found to be even smaller. These comparisons were made in Crump et al. (2008) without reoptimizing the likelihood. The authors note that reoptimization of the model subsequent to the variations would have made the fit of modified models even better.

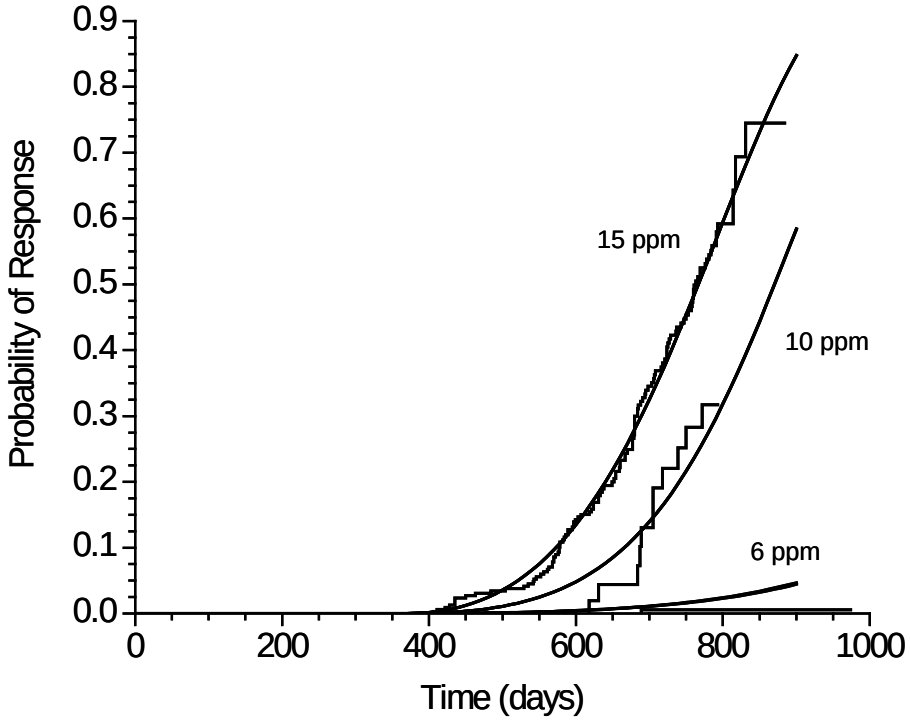


Figure B-34. Very similar model estimates of probability of fatal tumor in rats for three models in Figure B-32.

Note: The differences are visually indistinguishable. Models were derived from the implementation of Conolly et al. (2003) with the hockey-stick curves for αI(flux) and αN(flux) and variants derived from modifications (Mod 1 and Mod 2, Figure B-32) to αI(flux). Model probabilities are compared to K_m estimates. The three sets of model estimates are so similar that they cannot be distinguished on this graph.

Source: Crump et al. (2008)

The above modifications did not affect the basal rate of cell division in the model and likewise had no effect on the fit to the human background data (Crump et al., 2008).

Crump et al. (2008) noted that, although the threshold model for initiated cells in Conolly et al. (2003) was replaced with a model that had a small positive slope at the origin, the resulting curves, hockey-stick Mod 1 and hockey-stick Mod 2, could have been shifted slightly to the right along the flux axis in order to introduce a threshold for α_I without materially affecting the risk estimates resulting from these modified curves. Thus, "the assumption of a linear no-threshold response is not an essential feature of the modifications to the hockey-stick model; clearly threshold models exist that would produce essentially the same effect" (Crump et al., 2008).

Biological plausibility of alternate assumptions

Crump et al. (2008) provide many arguments to support the very small variations made to the α_I in Conolly et al. (2003) for their sensitivity analyses. These variations are found to be:

- consistent with the tumor-incidence data (Figure B-34);

- small compared with the variability and uncertainty in the cell replication rates characterized from the available empirical data (at the formaldehyde flux where α_I was varied);

- supported (qualitatively) by limited data, suggesting increased cell proliferation at doses below cytotoxic;

- perturbations to be expected on any dose response derived from laboratory animal data because of human population variability in cell replication (the Conolly et al. (2004) modeling assumes that the formaldehyde flux levels at which cell replication, normal and initiated, exceeds baseline rates remain essentially unchanged when extrapolated to the human.)

The analyses of the cell replication data show that the data are not consistently (over each site and time) indicative of a hockey-stick or J shape as the best representation of the data; in some cases, the data appear to be more representative of a monotonic increasing dose response without a threshold. This uncertainty is particularly prominent when examining the cell replication data at the 13-week exposure time and the pooled data from the PLM nasal site from Monticello et al. (1996) (B.2.2 "Characterization of uncertainty-variability in cell replication rates"). The earliest exposure time in this experiment was at 13 weeks; it is possible that early times are of more relevance to the carcinogenesis as well as for considering typical (frequent short duration) human exposures. Meng et al. (2010) measured cell replication in the anterior lateral meatus of the F344 rat using continuous labeling on rats exposed to all the concentration levels in the Monticello et al. (1996) experiment. Labeling index (i.e., LI, as opposed to ULLI in the Monticello experiment) was measured as the percentage of BrdU-labeled cells among the total number of cells counted at the nasal site. Their data are reproduced below in Figure B-35, where the asterisk denotes the observation of a statistically significant difference from the control group (Dunnett's test, $p < 0.01$). EPA determined that a linear regression provided good fits to all of the data ($R^2 = 0.97$) as well as to the subset of the data obtained by deleting the higher dose data at 10 and 15 ppm exposures ($R^2 = 0.84$). Thus, these data appear to be consistent with a monotonically increasing trend in the dose-response for cell replication.

For initiated cells, there are no data on which to evaluate the modifications made in Figures B-32 and B-33 to the assumption in equation B-13. However, some perspective can be gained by comparing them to the variability in the division rates obtained from the data on normal cells used to construct the formaldehyde model. As shown in Figure B-18 and discussed further in Subramaniam et al. (2008), these data show roughly an order of magnitude variation in the cell replication rate at a given flux. As part of a statistical evaluation of these data, a standard deviation of 0.32 was calculated for the log-transforms of individual measurements of division rates of normal cells (Crump et al., 2008). By comparison, the maximum change in the log-transform division rate of initiated cells resulting from hockey-stick Mod 2 was only 0.20, and the average change would be considerably smaller. Thus, although there are no data for initiated cells, it can be

said that the modifications introduced in Crump et al. (2008) for initiated cells are extremely small in comparison to the dispersion in the data for normal cells.

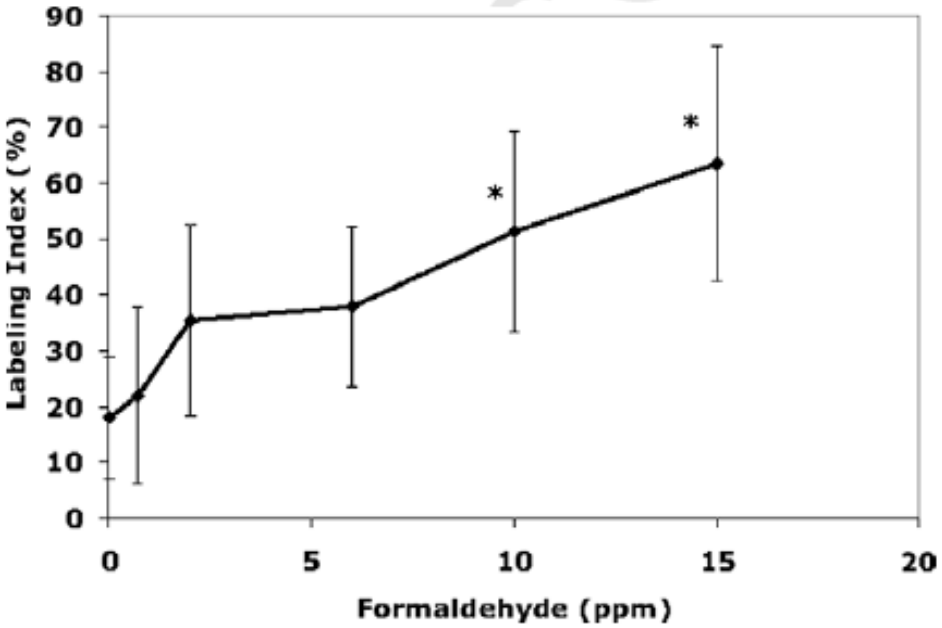


Figure B-35. Cell proliferation data from Meng et al. (2010).

The *y*-axis shows the percentage of BrdU-labeled cells among the total number of cells counted in the ALM section of the rat nose.
Reproduced with permission from Meng et al. (2010).

Effect of alternate assumptions for initiated cell kinetics on human risk estimates

Figure B-36 contains graphs of the additional human risks estimated [in Crump et al. (2008)] by applying these modified models for α_I and using all NTP controls, compared with those obtained by using the original Conolly et al. (2004) model. Each of the four modified models presents a very different picture from that of Conolly et al. (2004). At low exposures, these risks are three to four orders of magnitude larger than the largest estimates obtained by Conolly et al. (2004).

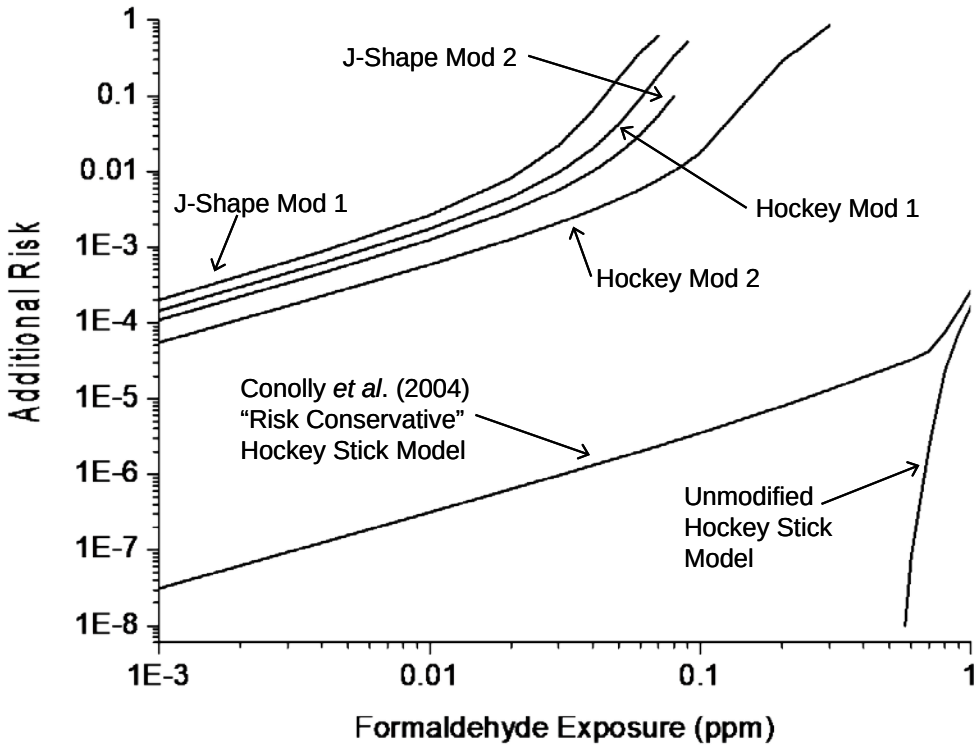


Figure B-36. Graphs of the additional human risks estimated by applying these modified models for α_I, using all NTP controls, compared to those obtained using the original Conolly et al. (2004) model.

Source: Crump et al. (2008).

These results have been criticized by Conolly et al. (2009) as being unrealistically large and above the realm of any epidemiologic estimate for formaldehyde SCC. Thus, they argue that the parameter adjustments made in Crump et al. (2008). are inappropriate. Crump et al. (2009) rebutted these points by arguing that the purpose of their work was not to provide a more reliable or plausible model but to carry out a sensitivity analysis. They argued that the changes made to the model (in their analyses) were reasonable because they did not violate any biological constraints or the available data. Further, they pointed out that "by appropriately mitigating the small modifications [they] made to the division rates of initiated cells, the model [would] provide any desired risk ranging from that estimated by the original model up to risks 1,000-fold larger than the conservative estimate in Conolly et al. (2004)."

Crump et al. (2008) also evaluated the assumption in equation D-3 of the CIIT modeling pertaining to initiated cell death rates (β_I) by making small changes to β_I. They report that they obtained similarly large values for estimates of additional human risk at low exposures. Obtaining reliable data on cell death rates in the nasal epithelium appears to be an unusually difficult proposition (Hester et al., 2003; Monticello and Morgan, 1997), and, even if data are obtained, they are likely to be extremely variable.

B.2.3. Estimates of Cancer Risk Using DNA Adduct Data from Animal Toxicology Studies and Background Incidence

DNA Adduct-Based Approach

Lu et al. (2010a) developed a highly sensitive MS method using [$^{13}CD_2$]-formaldehyde that reportedly distinguishes whether formaldehyde-induced hydroxymethyl-DNA monoadducts, in particular, the N^2-hydroxymethyl-dG (N^2-hmdG) adduct, originate from endogenous or exogenous sources of formaldehyde in rats and monkeys. In experiments using this technique, (Yu et al., 2015b; Lu et al., 2011; Moeller et al., 2011; Lu et al., 2010a) quantified these mono adducts formed from both sources in various tissues of rats and monkeys: nasal cavity, bone marrow, mononuclear white blood cells, spleen, thymus, tracheal bronchial lymph nodes, mediastinal lymph nodes, trachea, lung, kidney, liver, and brain. Swenberg et al. (2011) and Starr et al. (2016) used these adduct measurements and data on the background incidences of nasopharyngeal cancer, Hodgkin lymphoma, and leukemia in the U.S. population to develop cancer risk estimates by attributing the background incidences to endogenous formaldehyde, using the measured endogenous *N*2-hmdG adducts formed by formaldehyde in specific tissues as a biomarker of exposure. Their method, described by the authors as a "bottom-up approach" for risk estimation used the following steps:

1) DNA mono-adducts were used in the risk model as a marker of exposure (i.e., repairable) as opposed to a marker of effect (i.e., heritable mutations). While both adducts were reportedly formed by endogenous formaldehyde, only *N*2-hmdG adducts were detectable from exogenous formaldehyde.

2) Adducts formed endogenously were distinguished from those formed due to exogenous sources using $^{13}CD_2$-formaldehyde coupled with MS methods.

3) Endogenously and exogenously formed mono-adducts were measured in various tissues: nasal cavity, bone marrow, spleen, thymus, and mononuclear white blood cells (rats); nasal cavity, bone marrow (monkeys).

4) Adducts were measured in rats after one 6-hour exposure to 0.7, 2.0, 5.8, 9.1, and 15.2 ppm formaldehyde and five 6-hour exposures to 10 ppm, and in monkeys (cynomolgus macaques) after two 6-hour exposures to 2 and 6 ppm. There were no measurements carried out in unexposed animals. Time-course data were used to derive the half-life ($t_{1/2}$) for repair of the *N*2-hmdG adduct in rats.

5) No exogenous adducts were detected in any of the distant tissues (bone marrow, spleen, thymus, white blood cells); therefore, for these tissues the adduct levels were estimated by considering the limit of detection (LOD) of the method as an upper-bound estimate. This LOD was converted to the equivalent level of *N*2-hmdG adducts per 10^7 dG.

6) The risk model assumes a linear relation between cancer incidence and *N*2-hmdG adduct levels (used as an intracellular marker of exposure) over the concentration range of endogenous adducts. The same linear model is then assumed for exogenous adducts in order to carry out an upward extrapolation to low exposures (that are not high enough to

cause cytotoxicity). Unit risks for nasopharyngeal cancer (NPC), Hodgkin lymphoma (HL) and leukemia were calculated as follows:

a. Determine lower confidence limits on the endogenous *N*2-hmdG adduct levels measured in Step 3.
b. Assume the endogenous adduct level measured in rats to be the same in humans.
c. Convert exogenous *N*2-hmdG adduct levels from 6-hour exposure values to adduct levels to be expected under steady-state continuous exposure using the estimated $t_{1/2.}$
d. Assume adduct levels are a linear function of exposure (adduct) concentration, passing through the origin. Calculate the adduct per ppm ratio. Then, from c) above, calculate the continuous adduct level corresponding to 1 ppm.
e. Convert the continuous adduct level corresponding to 1 ppm exposure from rat to human by assuming that adduct levels scale in proportion to formaldehyde flux to the nasal tissue in each species. For the monkey, assume that humans receive the same levels of formaldehyde flux.
f. Consider endogenous and exogenous *N*2-hmdG adducts formed by formaldehyde to be biochemically indistinguishable (both were similarly related to low-dose formaldehyde carcinogenicity).
g. Use the U.S. population background lifetime incidence probabilities of NPC (7.25×10^{-4}), HL (2.3×10^{-3}), and leukemia (1.3×10^{-2}). Swenberg et al. (2011) consider values provided in the EPA draft assessment (for NPC) and the SEER Cancer Statistics Review (for HL and leukemia). Attribute these lifetime risks to the endogenous formaldehyde levels indicated by the adduct levels in step a (i.e., to the lower confidence limit on endogenous formaldehyde *N*2-hmdG adducts in the nose, bone marrow, or mononuclear white blood cells). Thus, calculate unit risk estimates for these specific cancers, expressed in units of risk per *N*2-hmdG adduct per 10^7 dG.
h. Using the unit risk estimates determined in Step g, calculate upper confidence limit on cancer risks for the continuous steady-state exogenous adduct level calculated in Step e, which corresponds to 1 ppm inhaled formaldehyde exposure concentration.

Swenberg et al. (2011) state that their risk estimates are conservative upper bounds on added lifetime risk at low environmental exposures, and cite the following reasons as support:

- The background risks of specific cancers are fully attributed to the internal dose represented by the endogenous *N*2-hmdG adducts measured in the corresponding tissue.
- Only *N*2-hmdG adducts are included (the unit risk would be lower if other higher endogenous adducts are included).
- A linear risk model is assumed.
- Exogenous adduct levels are assumed to be a linear function of exposure concentration, passing through the origin. The slope of this line is based on the mean adduct concentration at 10 ppm exposure which is an overestimate at low exposures because the actual relationship of adduct levels versus ppm is highly nonlinear (upwardly concave). This leads to a more conservative estimate for the cancer risk from step h of #7 above.
- The 95% lower confidence bound on mean adduct level is used, which can be assumed to correspond to the upper confidence bound on estimated risk.

- Monkeys appear to have lower exogenous N2-hmdG adduct levels than rats; therefore, risk estimates based on scaling rat adduct levels to humans in proportion to formaldehyde flux to nasal tissue would likely err on the side of being an over-estimate for humans.

EPA (Crump et al., 2014) evaluated the assumption in Swenberg et al. (2011) and Starr et al. (2016) that their use of a linear risk model necessarily yields an upper bound on the low-dose risk. The evaluation is elaborated further below.

By virtue of the additivity assumption (#6f), the effective dose to the DNA is represented by the total *N*2-hmdG adduct (endogenous plus exogenous) level. That is, the bottom-up approach allows the traditional dose-response curve (extra risk versus externally derived dose) to be rescaled so that the dose measure associated with zero external dose is now considered a positive dose equal to the levels found in tissues not exposed to an external source, and the line of zero extra risk is at a positive risk designated as the background risk. This is shown schematically in Figure B-37. The dashed line, showing the linearly extrapolated risk to exogenous exposures, is the central estimate of the linear slope based on the background risk P_0 of developing a specific cancer (attributed to an endogenous level of C_0). The solid curve represents a plausible true dose-response for a case in which the curve shapes upward in the (unobservable) endogenous range. It is reasonable to assume that the shape of the true dose-response curve is differentiable at the endogenous adduct level, and is concave upward at dose levels used in rodent bioassays (i.e., following typically used dose-response functions used in modeling the probability of tumor incidence, the slopes get steeper as dose increases and the second derivative is positive). Then it is clear from Figure B-37 that the bottom-up approach can never overestimate the relevant low-dose slope; any straight line between two points on the concave upward curve will underestimate the slope of the curve at the higher of the two doses. A similar argument can be made for a unit risk derived using a lower bound on C_0 to calculate an upper bound on P_0/C_0.

It is possible, nonetheless, that the extent of underestimation discussed above (that is, from a "bottom up" linear fit to a dose-response curve) can be offset by the conservatism in attributing all cancers of the specified type to the endogenous dose. However, this is difficult to assess. If one focuses only on the specified type of tumor, the assumption on its own appears to be conservative. It is not, however, easy to ascertain whether that degree of conservatism would be greater than the under-estimation. In addition, the selection of the type of cancer is informed by, and thus dependent on, higher dose data. To the extent the higher dose data did not detect other types of cancer, the attribution of all observed cases of the selected tumor may not capture all the relevant cases.

Furthermore, the slope of increased risk with increasing adduct levels may not be linear even over the range of the endogenous adducts; the slope may be concave upward as endogenous defensive mechanisms become less effective in dealing with endogenous adduct levels as adduct levels increase over the endogenous range. This seems a plausible scenario, as organisms would have evolved some level of defensive mechanisms to deal with endogenous levels of adducts, yet

there is an energy cost associated with over-capacity; thus, these defensive capabilities are not fully effective over the entire endogenous range, and this is consistent with the observance of "background" rates of cancer. Under this plausible scenario, the actual slope of the adduct-based unit risk estimate at the lower confidence bound on the mean endogenous *N*2-hmdG adduct level may be substantially higher than that suggested by a linear relationship over the endogenous range and, thus, the slope obtained from the linear assumption does not necessarily provide an upper bound on risk.

It may be noted that the bottom up approach is not consistent with the concept of additivity to background disease processes on the basis of which local linearity in the proximity of zero exogenous dose is thought to be reasonable. The approach requires a linear dose response below zero exogenous dose which is not required to assume additivity to background.

An additional uncertainty arises from the observation that while endogenous *N*2-hmdG and *N*6-hmdA adducts were both measured in rat and monkey nasal tissues, inhalation of formaldehyde resulted in a concentration-related pattern for exogenous *N*2-hmdG adducts only, and no detectable exogenous *N*6-hmdA adducts. If these differences (in regards the observation of *N*6-hmdA versus *N*2-hmdG adducts) are attributable to differences in the effects of endogenous versus exogenous formaldehyde in inducing DNA adducts, it is not clear that one can assume (as in 6f) additivity of endogenous and exogenous formaldehyde.

In general, it does not appear to be possible to characterize the results using this approach as providing a conservative upper bound on cancer risk. Notwithstanding this limitation, the bottom-up approach in Swenberg et al. (2011) and Starr et al. (2016) is particularly attractive when other phenomena such as significant cytotoxicity and subsequent impact on DNA repair prior to mutations are occurring at higher doses. Because the approach does not use the higher-dose data (other than to identify the type of tumors of concern for analysis), it provides a unique perspective on risk estimates derived from these data.

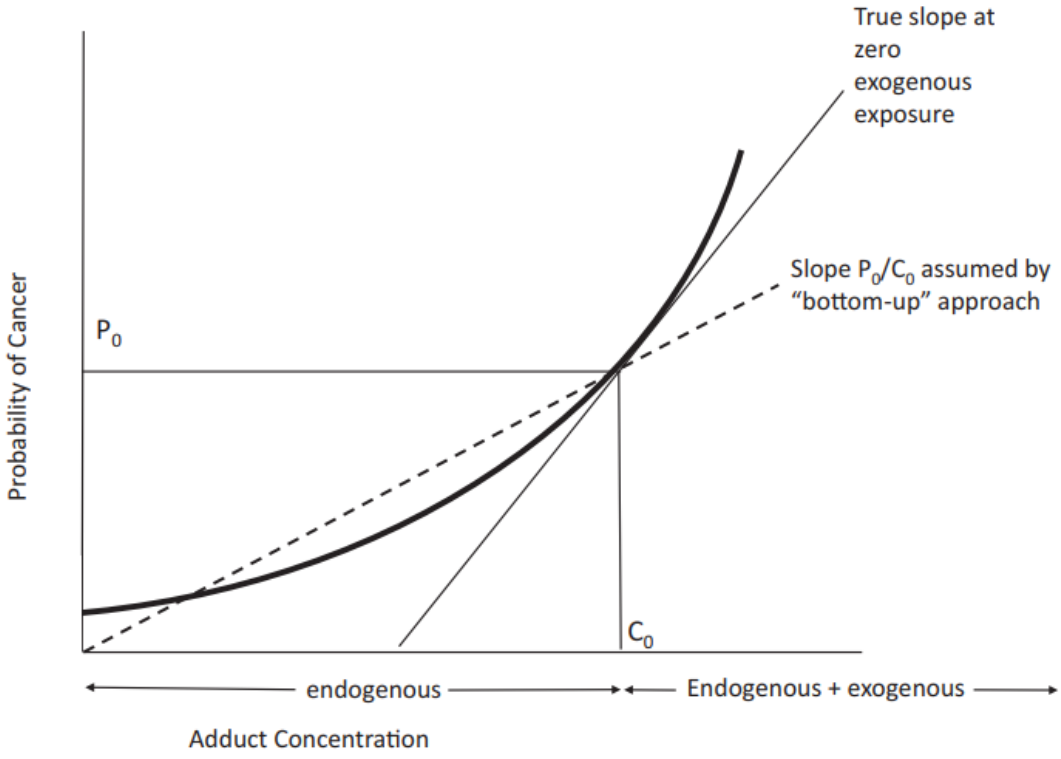


Figure B-37. Schematic of the bottom-up approach

Source: Adapted from (Crump et al., 2014)

APPENDIX C. ASSESSMENTS BY OTHER NATIONAL AND INTERNATIONAL HEALTH AGENCIES

Table C-1. Hazard conclusions and toxicity values developed by other national and international health agencies

Organization	Conclusions and toxicity values
Agency for Toxic Substances and Disease Registry (ATSDR, 1999)	Chronic inhalation minimal risk levels (MRL) = 0.008 ppm using a composite uncertainty factor (UF) of 30, based on clinical symptoms of irritation of eyes and upper respiratory tract and mild damage to the nasal epithelium in chronically exposed workers (Holmstrom et al., 1989c); Intermediate MRL = 0.03 ppm using composite UF of 30 based on nasopharyngeal irritation in Cynomolgus monkeys (Rusch et al., 1983); Acute MRL = 0.04 ppm using UF = 9 based on nasal and eye irritation in human volunteers (Pazdrak et al., 1993).
Interim Acute Exposure Guideline Levels (AEGLs) for Formaldehyde, National Advisory Committee for AEGLs for Hazardous Substances (NAC/AEGL, 2008)	AEGL-1 (nondisabling)—0.90 ppm (1.1 mg/m^3) for exposures ranging from 10 min to 8 hr to protect against mild irritation, based on mild irritation in human subjects. AEGL-2 (disabling)—14 ppm (17 mg/m^3) for exposures ranging from 10 min to 8 hr to protect against mild lacrimation with adaptation in humans. AEGL-3 (lethal)—100 ppm (123 mg/m^3) for a 10-min exposure to 35 ppm (43 mg/m^3) for an 8-hr exposure, the highest nonlethal values in the rat.
National Toxicology Program (NTP, 2011)	Known to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in humans (consistent findings for nasopharyngeal, sinonasal, and myeloid leukemia) and supporting data on mechanisms of carcinogenesis (NTP, 2011).
National Institute of Occupational Safety and Health (NIOSH, 2011, https://www.cdc.gov/niosh/idlh/50000.html)	Potential occupational carcinogen. Recommended exposure limit (REL)—0.016 ppm (0.04 mg/m^3) TWA for up to a 10-hr workday and a 40-hr work wk.
Occupational Safety and Health Standard 1910.1048	Permissible exposure limit (PEL) for general industry—0.75 ppm (0.92 mg/m^3) TWA for an 8-hr workday; Short-term exposure limit: 2 ppm (2.5 mg/m^3), 15-min duration.
International Agency for Research on Cancer, Monograph Vol. 88 (IARC, 2006); Monograph Vol. 100F (IARC, 2012)	Sufficient evidence in humans for the carcinogenicity of formaldehyde based on nasopharyngeal cancer and leukemia (Group 1). Sufficient evidence in experimental animals for the carcinogenicity of formaldehyde.
European Union, European Commission, Scientific Committee on Occupational Exposure Limits (SCOEL, 2017)	Carcinogen group C: genotoxic carcinogen with a mode-of-action-based threshold. Occupational exposure limit (OEL)—8h-TWA of 0.3 ppm (0.369 mg/m3); STEL 15 min of 0.6 ppm (0.738mg/m3) based on cytotoxic irritation in studies of human volunteers.
Health Canada (2006, https://www.canada.ca/en/health-canada/services/publications/healthy-living/residential-indoor-air-quality-guideline-formaldehyde.html) Residential Indoor Air Quality Guideline	Short-term exposure: 123 µg/m^3 (1-hr average) based on eye, nose, and throat irritation (Kulle, 1993); long-term exposure: 50 µg/m^3 (8-hr average) based on respiratory symptoms in children with asthma (Rumchev et al., 2002).

Organization	Conclusions and toxicity values
(Health Canada, 2001) Priority Substances List Assessment Report	The inhalation of formaldehyde under conditions that induce cytotoxicity and sustained regenerative proliferation is considered to present a carcinogenic hazard to humans.

APPENDIX D. 2011 NATIONAL RESEARCH COUNCIL EXTERNAL PEER REVIEW COMMENTS ON THE 2010 DRAFT AND EPA'S DISPOSITION

This section itemizes the comments and recommendations regarding the June 2010 draft toxicological review of formaldehyde that was released for external peer review by a committee of the National Research Council (NRC). The report by the NRC committee was sent to the EPA in 2011. In light of the substantive recommendations to adopt a more systematic approach to the assessment, the development of the current assessment involved a fresh start (from scratch), and now includes more explicit rationales and criteria for decisions, and thorough documentation of all steps in the process from the literature search through the development of toxicity values. Thus, this is a completely different document. Although the comments from the NRC may not be directly applicable to the current assessment, many of the issues that were raised remain pertinent, and responses were developed to address the comments that were received on the prior draft's contents.

D.1. NRC FORMALDEHYDE PANEL SUMMARY RECOMMENDATIONS SPECIFIC TO FORMALDEHYDE AND EPA RESPONSES

<u>General Recommendations (NRC comment bullets) From Executive Summary and Chapter 7</u>

- Rigorous editing is needed to reduce the volume of the text substantially and address the redundancies and inconsistencies; reducing the text could greatly enhance the clarity of the document.

  Response: EPA has taken steps to reduce the amount of text and to display relevant information more clearly and succinctly in tables and graphs. The hazard identification section has been reorganized to describe the human and animal evidence together by health hazard. An integrated weight of evidence (evidence integration) section for each hazard is now included to enhance clarity. Repetition is minimized and all summaries and conclusions have been carefully reviewed and edited to prevent inconsistency.

- Chapter 1 of the draft assessment needs to discuss more fully the methods of the assessment, including a description of search strategies used to identify studies with the exclusion and inclusion criteria clearly articulated and a better description of the outcomes of the searches (a model for displaying the results of literature searches is provided later in this chapter) and clear descriptions of the weight-of evidence approaches used for the various noncancer outcomes. The committee is recommending not the addition of long descriptions of EPA guidelines but rather clear concise statements of criteria used to exclude, include, and advance studies for derivation of the RfCs and unit risk estimates.

Response: The new Preface to the toxicological review (and supporting Appendices) describes the approaches used to identify relevant studies and the process through which specific studies were reviewed for hazard identification and selected for use in derivation of toxicity values. Because literature searches were conducted for each health hazard independently, the databases, search strings, inclusion and exclusion criteria and diagrams displaying results are presented by health hazard in the supplemental materials with a summary included for each health hazard. A framework developed for evaluating weight of evidence (evidence integration) for noncancer effects is also transparently described in the new Preface. These methods for the assessment, which was developed de novo after the NRC peer review in 2011, served as the foundation for the IRIS standard operating procedures for developing IRIS assessments (U.S. EPA, 2020), which were reviewed by the National Academy of Sciences, Engineering, and Medicine (NASEM) (NASEM, 2021).

- Standardized evidence tables that provide the methods and results of each study are needed for all health outcomes; if appropriate tables were used, long descriptions of the studies could be moved to an appendix or deleted.

Response: EPA has developed tables to summarize the studies in humans and animals that were used to synthesize the evidence for specific endpoints and reduced the amount of text that simply describes studies.

- All critical studies need to be thoroughly evaluated with standardized approaches that are clearly formulated and based on the type of research, for example, observational epidemiologic or animal bioassays. The findings of the reviews might be presented in tables to ensure transparency.

Response: EPA implemented these suggestions and applied a framework for systematic review for the review of epidemiology and toxicology studies of formaldehyde inhalation relevant to each considered hazard. The studies identified as meeting the PECO criteria were evaluated for their ability to inform the hazard reviews using standardized approaches and were categorized by a level of confidence (*high*, *medium*, *low*, and *not informative*). The issues pertinent to evaluating the strengths and limitations of individual studies with respect to specific health endpoints are discussed, and each study evaluation is documented in tables found in the supplemental material for each health hazard. The results of the study evaluations (e.g., confidence) are included in the evidence tables that summarize the studies found in each hazard section. Studies identified as *not informative* are not included in the evidence tables and do not contribute to hazard identification or dose-response decisions; these excluded studies are identified (e.g., in the discussion of methods in each section; in the study evaluation tables in the supplemental material). A simplified evaluation process was applied to mechanistic studies informing potential mode of action for respiratory effects and genotoxic endpoints (epidemiology studies for genotoxicity) and tables documenting the evaluations are found in the supplemental materials.

- The rationales for selection of studies that are used to calculate RfCs and unit risks need to be articulated clearly. All candidate RfCs should be evaluated together with the aid of graphic displays that incorporate selected information on attributes relevant to the database.

Response: The rationale for selecting studies for RfCs derivation are presented in the Preface to the assessment and in Chapter 2 of this toxicological review. An array of the studies and the candidate values, including key uncertainties, was developed and discussed to clearly present and justify the information and rationales used by EPA in developing the RfC.

- The weight-of-evidence descriptions need to indicate the various determinants of "weight." The reader needs to be able to understand what elements (such as consistency) were emphasized in synthesizing the evidence.

Response: The methods for synthesizing evidence and developing evidence integration judgments for each unit of analysis and health effect category, including specific considerations regarding causality that can either increase or decrease certainty in the available evidence, are described in the Preface to the toxicological review. Assessment development was based on EPA guidelines and standard IRIS procedures (U.S. EPA, 2020).

- "In general, the committee found that the draft was not prepared in a consistent fashion; it lacks clear links to an underlying conceptual framework; and it does not contain sufficient documentation on methods and criteria for identifying evidence from epidemiologic and experimental studies, for critically evaluating individual studies, for assessing the weight of evidence, and for selecting studies for derivation of the RfCs and unit risk estimates" (pp. 3–4).

Response: As described for the above comments, the current toxicological review follows a unifying conceptual framework, which is followed and documented throughout for identifying the evidence, evaluating individual studies, synthesizing the evidence within and across evidence streams, and for deriving organ- or system-specific RfCs, the overall RfC, and unit risk estimates.

Toxicokinetics

- The committee agrees with EPA's conclusion that "certain formaldehyde-related effects have the potential to modulate its uptake and clearance" (U.S. EPA, 2010), pp. 3–5}. Some of the effects, such as changes in mucociliary function and altered nasal epithelium, could occur in humans. However, reflex bradypnea and related modulating effects seen in rodents do not occur in phylogenetically higher animals (nonhuman primates) or humans. Thus, formaldehyde exposures at concentrations relevant for an RfC or unit risk are unlikely to alter its toxicokinetics.

Response: Consistent with the comment by the committee, the current draft assessment does not argue that the reflex braypnea-related effects are relevant for an RfC or unit risk. The study results on changes in mucociliary clearance are discussed in the supplemental materials and changes in nasal epithelium are discussed in respiratory pathology hazard section. These discussions examine the concentration and duration relationships observed for formaldehyde. Reflex bradypnea in experimental animals is discussed if relevant to the interpretation of the results of toxicology studies (generally, as a confounder).

- Formaldehyde has also been measured in exhaled breath, but the interpretation of some measurements made with mass spectrometry has been questioned (Schripp et al., 2010; Spanel and Smith, 2008). Spanel and Smith (2008) showed that a trace contaminant (up to

1%) of the reagent gas used in real-time mass-spectrometric methods—specifically proton-transfer reaction mass spectrometry (PTRMS) and selected ion flow tube mass spectrometry (SIFT-MS)—reacts with endogenous methanol and ethanol that is normally found in exhaled breath to produce the same main ion (mass-to-charge ratio of 31) as is used to measure formaldehyde. Thus, they concluded that up to 5 ppb of the formaldehyde concentration determined in the exhaled breath of humans reported in earlier studies that did not account for this confounding may be due to methanol or ethanol and not formaldehyde; that is, 1% of total background concentrations of methanol or ethanol of about 500 ppb would be misclassified as formaldehyde. The committee concurs with EPA's concerns as to whether some published exhaled breath measurements of formaldehyde are analytically valid. The committee also notes that this methodologic problem is inconsistently addressed by EPA in its reanalysis of the exhaled-breath experiments. The committee concludes, however, that regardless of the methodologic issue related to breath analysis, formaldehyde is normally present at a few parts per billion in exhaled breath after the measurement error associated with a trace contaminant in the reagent gas used in previous mass spectrometric methods is taken into account.

Response: It is difficult to say what range of formaldehyde concentration may be found in exhaled breath, although levels are likely to be very low. Subjects in several of the cited studies were inhaling formaldehyde at concentrations of about 10 ppb, so the inhaled air contributed to the measurements of formaldehyde in exhaled air.

A study by Riess et al. (2010), published shortly after the NAS review commenced, was not hindered by the limitations of previous studies. All subjects in this study inhaled formaldehyde-free air. No formaldehyde could be detected in exhaled breath of any subjects, including smokers, using a method with a limit of detection of <0.5 ppb.

Regardless of the technical limitations in the studies, the toxicity values derived in the toxicological review are intended to protect the population from the extra risk imposed by inhalation of formaldehyde in the air.

- The committee concludes that formaldehyde is an endogenous compound and that this finding complicates assessments of the risk posed by inhalation of formaldehyde. The committee emphasizes that the natural presence of various concentrations of formaldehyde in target tissues remains an important uncertainty with regard to assessment of the additional dose received by inhalation.

Response: The current assessment estimates the risk over background that results from only the exogenous exposure and assumes that the background incidence of cancer or other health hazards already includes risk that may potentially be attributed to endogenous formaldehyde. However, as discussed in the assessment in the context of conclusions from dosimetry models that accounted for endogenous tissue concentrations, the natural presence of formaldehyde in target tissues does contribute to uncertainty in extrapolating the dose-response of formaldehyde to very low exposures. Additionally, endogenous levels of formaldehyde are highly variable in humans, and some individuals are deficient in the detoxifying enzymes. These issues are discussed in the Preface, Sections 1.1.3, 1.4.1 and 2.2, and Appendix A.2.1.

- The draft IRIS assessment of formaldehyde provides an exhaustive discussion of formaldehyde toxicokinetics, carcinogenic modes of action, and various models. Although

the committee agrees with much of the narrative, several issues need to be addressed in the revision of the draft assessment. First, there is broad agreement that formaldehyde is normally present in all tissues, cells, and bodily fluids and that natural occurrence complicates any formaldehyde risk assessment. Thus, an improved understanding of when exogenous formaldehyde exposure appreciably alters normal endogenous formaldehyde concentrations is needed.

Response: The current assessment discusses the studies that evaluated formaldehyde concentrations in upper respiratory tract tissues and blood after formaldehyde inhalation in rodents (see the toxicokinetics summary Chapter 1 of the toxicological review and additional details in Appendix A.2). The studies concluded that DPX in bone marrow associated with inhaled formaldehyde were the result of metabolic incorporation of the inhaled formaldehyde in the nasal tissues, not from distribution and direct interactions with the aldehyde in bone marrow (Casanova-Schmitz et al., 1984b; Casanova-Schmitz and Heck, 1983). In addition, the assessment discussed the research using sophisticated measurements of hydroxymethyl DNA adducts differentiating between inhaled and endogenous formaldehyde in the upper respiratory tract, blood and other organs (Leng et al., 2019; Lai et al., 2016; Yu et al., 2015b; Swenberg et al., 2013; Lu et al., 2011; Moeller et al., 2011; Swenberg et al., 2011; Lu et al., 2010a). These studies did not find evidence that inhaled formaldehyde is distributed substantially beyond the respiratory tract tissues. Although there are remaining uncertainties regarding the extent that inhaled formaldehyde is distributed, the lack of systemic distribution is an assumption used in the assessment to provide a framework for presenting and interpreting the evidence concerning the potential hazards of formaldehyde inhalation.

- One approach that EPA could use would be to complete an analysis of variability and uncertainty in measuring and predicting target-tissue formaldehyde concentrations among species. Only with such an analysis can one begin to identify and address openly and transparently the question of how much added risk for an endogenous compound is acceptable.

Response: This assessment does not make judgments as to whether any specific added risk is acceptable, decisions which are made by policymakers under federal, state, and other regulatory authorities. The conclusions about potential health impacts are derived from evaluating the relationships in available studies between different inhaled concentrations of formaldehyde and observed health effects. As mentioned earlier, results in Schroeter et al. (2014) are consistent with the assumption that inhaled formaldehyde at relevant concentrations adds to mean endogenous concentrations in nasal tissue.

We agree that more data on the variability of endogenous formaldehyde concentrations among individuals would be useful to the discussion. The individual animal data on DNA adducts formed by formaldehyde in Swenberg et al. (2013), kindly made available to EPA by the authors, are a good example in this regard. A number of animals in these data had very high endogenous levels of these adducts; in these animals, even at a low inhaled exposure concentration of 2 ppm, the total (endogenous plus exogenous) internal dose, as measured by the level of DNA adducts, was comparable to the mean total internal dose measured in the group of animals exposed at 10 ppm (a dose at which considerable carcinogenicity was observed in animal bioassays). Heck and co-workers found the variability in endogenous levels to be greater than the difference between mean endogenous and exogenous levels in nasal tissues of multiple species at the lowest exposure levels in their studies (see Appendix

A.2.7). However, these data are from a small sample, and data from other studies (Swenberg et al., 2013) suggest that the population variability in endogenous levels, and the variation in endogenous levels across tissues, is likely to be large. Some individuals are thought to be deficient in their capacity to detoxify endogenous formaldehyde (Dingler et al., 2020), and may therefore be particularly susceptible to the exogenous exposure.

- A series of studies using dual-labeled (14C/3H) formaldehyde in rats has been performed to address the analytic concern (Casanova-Schmitz et al., 1984b; Casanova-Schmitz and Heck, 1983). The draft IRIS assessment accurately summarizes the main conclusions reached from those experiments, namely that "labeling in the nasal mucosa was due to both covalent binding and metabolic incorporation," that "DPX [were] formed at 2 ppm or greater in the respiratory mucosa," and that "formaldehyde did not bind covalently to bone marrow macromolecules at any exposure concentration" (up to 15 ppm) (U.S. EPA, 2010, pp. 3–12, pp. 3–12). The labeling of bone marrow macromolecules was found by the investigators to be due entirely to metabolic incorporation of the radiolabels, not to direct covalent binding of intact formaldehyde. The committee views those findings as supporting the hypothesis that inhaled formaldehyde is not delivered systemically under the exposure conditions used in the studies (0.3–15.0 ppm, 6 hr) (U.S. EPA, 2010).

  Response: The current assessment concludes that, although uncertainties remain regarding the extent that inhaled formaldehyde is distributed, the lack of systemic distribution is sufficiently supported, and this is used as an assumption in the assessment to provide a framework for presenting and interpreting the evidence concerning the potential hazards of formaldehyde inhalation.

- The committee also found that the more contemporary work performed by Lu et al. (2010a) that examined formaldehyde-induced DNA adducts and DDX cross links provided no direct evidence of systemic availability of inhaled formaldehyde. The Lu et al. (2010a) study used 13CD2-labeled formaldehyde and showed that 13CD2-formaldehyde-DNA adducts and DDX were confined to the nasal cavity of exposed F344 rats, even though they examined much more DNA isolated from bone marrow, lymphocytes, and other tissues at distant sites for the adducts. The male Fischer 344 rats were exposed to [13CD2]-formaldehyde at 10 ppm for 1 or 5 days (6 hr/d) with a single nose-only unit.

  Response: Lu et al. (2010a) is discussed in the current draft assessment draft, along with more recent studies confirming and expanding these observations (Leng et al., 2019; Lai et al., 2016; Yu et al., 2015b; Lu et al., 2011). EPA agrees that this study shows that the formaldehyde monoadducts and DNA-DNA cross links are detectable in nasal cavity, but not in bone marrow, of exposed rats. EPA agrees that this study does not provide evidence that formaldehyde is transported to bone marrow.

- The strongest data cited by EPA in support of systemic delivery of inhaled formaldehyde come from several studies in which antibodies to formaldehyde-hemoglobin and formaldehyde-albumin adducts were detected in blood from exposed workers, smokers, and laboratory animals. The studies did not definitively demonstrate, however, whether adduct formation occurs at a site distant from the portal of entry. For example, it is not known whether the adducts could be formed in the airway submucosal capillary beds or reflect systemic delivery of formaldehyde. Moreover, the draft IRIS assessment does not evaluate the antibody work as critically as the direct chemical-analysis approaches. The committee found that the draft does not offer a sufficient basis for EPA's reliance on the

antibody data to support the hypothesis that formaldehyde (or its hydrated form, methanediol) may reach sites distal to the portal of entry and produce effects at those sites.

Response: Whether the antibodies detected in the blood indicated adducts formed in airway submucosal capillary beds or in the blood is an uncertainty that is acknowledged in the current draft assessment. All discussions in the toxicological review follow from the premise that the evidence base does not support the hypothesis that the observed effects of inhaled formaldehyde are due to its delivery (in any intact form, including its hydrated form, methanediol) to systemic organs. These studies are discussed in the section on possible modes of action for lymphohematopoietic cancers (Section 1.3.3 of the toxicological review).

- Questions have arisen regarding the possibility that formaldehyde reaches distal sites as methanediol. However, although equilibrium dynamics indicate that methanediol would constitute more than 99.9% of the total free and hydrated formaldehyde, the experimental data described above provide compelling evidence that hydration of formaldehyde to methanediol does not enhance delivery of formaldehyde beyond the portal of entry to distal tissues. Furthermore, Georgieva et al. (2003) used a pharmacokinetic modeling approach that explicitly accounted for the competing processes of hydration, dehydration, diffusion, reactivity with macromolecules, and metabolism and demonstrated that hydration-dehydration reaction rates determined from equilibrium studies in water are not applicable in biologic tissues, given that their use in the model resulted in simulations that were inconsistent with the available data. For example, the calculated dehydration rate from equilibrium dynamics studies in water was so small relative to other competing rates that too little formaldehyde would be available to account for the measured DPX rates. Thus, the data provide a strong indication that the hydration-dehydration reaction should not be rate-limiting and can thus be ignored in modeling the disposition of inhaled formaldehyde in nasal tissues.

  Response: EPA agrees that the hydration-dehydration reaction is not likely to play a significant role in the disposition of formaldehyde following absorption into nasal tissue. This is reflected in the analyses presented in the current draft.

- EPA also suggested that systemic delivery of formaldehyde-glutathione adducts and latter release of free formaldehyde may result in delivery of formaldehyde to sites distal to the respiratory tract. However, experimental data supporting that hypothesis are lacking, as acknowledged by the draft IRIS assessment. In fact, additional data based on even more sensitive analytic methods published since the draft assessment was released casts further doubt on the hypothesis that formaldehyde reaches the systemic distribution in a form that can react with macromolecules in tissues remote from the portal of entry (Lu et al., 2011; Moeller et al., 2011; Swenberg et al., 2011).

  Response: EPA agrees that the hypothesis of GSH-mediated delivery of formaldehyde lacks experimental support. The current draft assessment includes the studies by Lu et al. (2011), Moeller et al. (2011), Swenberg et al. (2011), Yu et al. (2015b), and the more recent report by Lai et al. (2016) and Leng et al. (, 2019, 6113745}.

- The committee also found two divergent statements regarding systemic delivery of formaldehyde in the draft IRIS assessment. Some parts of the draft assume that the high reactivity and extensive nasal absorption of formaldehyde restrict the systemic delivery of

inhaled formaldehyde to the upper respiratory tract (for example, for example, U.S. EPA, 2010, pp. 4–371, pp. 4–371). Under that assumption, systemic responses—including neurotoxicity, reproductive toxicity, and leukemia—are unlikely to arise from the direct delivery of formaldehyde (or methanediol) to a distant site in the body, such as the brain, the reproductive tract, and the bone marrow. Other portions of the document presume systemic delivery of formaldehyde (or its conjugates) and use this presumption to account in part for the systemic effects (see, for example, p. 4-1, lines 16-19; p. 4-472, line 18; Section 4.5.3.1.8; and p. 6-23, line 31). The committee found the inconsistency to be troubling, and the divergent assumptions are not justified.

Response: All discussions in this draft toxicological review follow from the premise that the evidence base does not support the hypothesis that the observed effects of inhaled formaldehyde are due to its delivery (in any intact form, including its hydrated form, methanediol) to systemic organs.

- The committee concludes that the issue of whether inhaled formaldehyde can reach the systemic circulation is extremely important in assessing any risk of adverse outcomes at nonrespiratory sites associated with inhalation of formaldehyde. Moreover, the committee concludes that the weight of evidence suggests that it is unlikely for formaldehyde to appear in the blood as an intact molecule, except perhaps after exposures at doses that are high enough to overwhelm the metabolic capability of the tissue at the site of entry. Thus, although many sensitive and selective investigative approaches have been used, systemic concentrations from inhaled formaldehyde are indistinguishable from endogenous background concentrations. The committee, however, notes the importance of differentiating between systemic delivery of formaldehyde and systemic effects. The possibility remains that systemic delivery of formaldehyde is not a prerequisite for some of the reported systemic effects seen after formaldehyde exposure. Those effects may result from indirect modes of action associated with local effects, especially irritation, inflammation, and stress.

  Response: EPA agrees with NAS that systemic delivery is not a prerequisite for systemic effects. EPA also agrees with NAS that the systemic effects could be due to indirect or unknown mode(s) of action. EPA conducted a systematic evaluation of the evidence pertinent to possible mechanistic events responsible for the observed respiratory effects identified in the toxicological review. Some of these events related to irritation, inflammation, and oxidative stress may also be relevant to effects observed at distal sites, and this evidence is included in the MOA discussions for systemic effects, including myeloid leukemia, in the current toxicological review.

- Inhaled formaldehyde, a highly reactive chemical, is absorbed primarily in the upper airways and remains predominantly in the respiratory epithelium. The weight of evidence indicates that formaldehyde probably does not appear in the blood as an intact molecule except at doses high enough to overwhelm the metabolic capability of the exposed tissue. The draft IRIS assessment presents divergent opinions regarding the systemic delivery of formaldehyde that need to be resolved.

  Response: The current assessment presents a consistent view on the evidence regarding the distribution of formaldehyde. All discussions in this draft toxicological review follow from the premise that the evidence base does not support the hypothesis that the observed effects of inhaled formaldehyde are due to its delivery to systemic organs.

- In rewriting the sections of the draft IRIS assessment that pertain to the topics reviewed in this chapter, EPA should consider the implications of the most recent work. References to older studies on DNA-adduct measurements may need to be reanalyzed in light of the most recent analytic techniques that achieved superior sensitivity (for example, for example, Lu et al., 2010a). In particular, the committee finds the recent study of Lu et al. (2010a) to be highly informative and the first one to distinguish clearly between exogenous and endogenous formaldehyde-induced DNA adducts. Although the study does not challenge the notion that DNA adducts play only a minor, if any, role in formaldehyde genotoxicity and carcinogenicity, compared with DNA-protein cross links, it adds to the evidence of the inability of formaldehyde to reach distant sites. Likewise, the positive study by Wang et al. (2009a) is not adequately described in the draft IRIS assessment, nor is it clear to the committee why so much emphasis is placed on the study by Craft et al. (1987) (pp. x and 45 [mode of action]).

  Response: The studies by Lu et al. (2010a), Wang et al. (2009a), and Craft et al. (1987) are described and evaluated in the current draft, along with more recent studies (see Appendix A.4), and strengths and limitations are clearly presented.

Dosimetry modeling of formaldehyde

- The CFD models were fairly evaluated and that the sources of uncertainty in dose metrics used in dose-response assessments were appropriately treated. [pp 31]

- The committee disagrees with EPA's findings that CFD models are not useful for low-dose extrapolations. In fact, flux results from the CFD models can easily be scaled from an exposure of 1 ppm—as given by Kimbell et al. (2001b; 2001) and Overton et al. (2001)—to lower concentrations because of the linear flux-concentration relationship that was used by the authors. Therefore, the committee recommends that the CFD-based approach also be used to extrapolate to low concentrations, that the results be included in the overall evaluation, and that EPA explain clearly its use of CFD modeling approaches (p. 31).

  Response: EPA agrees with the committee that "flux results from the CFD models can easily be scaled from an exposure of 1.0 ppm to lower concentrations because of the linear flux-concentration relationship that was used by the authors of the model," and has used this approach in the assessment. As explained further in response to questions on EPA's use of BBDR modeling, the assessment presents rat and human risk estimates based on the BBDR modeling. This modeling used CFD model calculations as input. Because BBDR-predicted values differ from each other by many orders of magnitude, EPA's calculation of unit risk is based on straight line extrapolation from points of departure, derived using different implementations of the BBDR model in the rat. Extrapolation to the human is then based on CFD model-derived wall-mass flux estimates in the rat and human nose.

- The committee notes that the CFD models of Kimbell et al. (2001b; 2001) do not account for potential effects of sensory irritation on ventilation inasmuch as only two mass-transfer coefficients, one for mucus-coated and one for non-mucus-coated epithelial regions of the nose, were used in all simulations to derive uptake into nasal tissues. However, later models that account for DPX cross links and cytotoxicity (Conolly et al., 2004, 2003; Georgieva et al., 2003; Conolly, 2002; Conolly et al., 2000) relied on animal data that were obtained at concentrations that potentially caused irritation to derive parameters

associated with metabolism and reactivity; thus, the potential effect of altered ventilation was indirectly compensated for in those model simulations.

Response: EPA agrees with the committee. The statement on uncertainty in model (BBDR and DPX) structure associated with effects of sensory irritation on ventilation has been deleted from the current draft assessment.

- The draft IRIS assessment raises the criticism that the nasal CFD models are based on a single geometry for each species. Thus, the models do not address variability that arises from differences in airway anatomy. A recent paper by Garcia et al. (2009) evaluated the effect of individual differences in airway geometry on airflow and uptake of reactive gases, such as formaldehyde. Although the sample was small (five adults and two children), the individual differences in airway geometry alone caused the potential flux rates to vary by a factor of only 1.6 over the entire nose and by a factor of 3–5 at various distances along the septal axis of the nose. The committee agrees with EPA that although the sample was small, the estimates of individual variability are consistent with default uncertainty factors applied to internal dose metrics that account for human variability.

Response: For noncancer effects, EPA has used an uncertainty factor to address human variability. For cancer effects, EPA does not apply uncertainty factors for intrahuman variability but recognizes that there is uncertainty in estimates of unit risk.

Biology-based dose-response (BBDR) modeling of rat nasal tumors

- The committee agrees that [EPA's] sensitivity analysis added value to the interpretation of the Conolly et al. models (p. 36). The committee also acknowledges that the draft IRIS assessment provides a thorough review of the BBDR models, the major assumptions underpinning the extrapolation to humans, and EPA's own series of papers that evaluated the sensitivity of the BBDR models to these assumptions even though the committee may not agree with the validity of all the resulting manipulations (p. 42).

- EPA's reanalysis was consistent with its cancer guidelines that specify that the uncertainties and variability in model parameters must be understood and articulated so that predictions of adverse responses and extrapolations to human exposures can be appropriately characterized from the standpoint of human health protection (p. 36).

- The committee questions the degree to which manipulations of the range of model parameter values can and should be performed to reflect potentially divergent outcomes (p. 36). The committee is concerned about the possibility that those adjustments of the Conolly et al. models may not be scientifically defensible (p. 43).

- EPA, on the basis of extreme alternative model scenarios, chose not to use the BBDR models developed by Conolly et al. (2004, 2003); however, the committee questions the validity of some of these scenarios (p. 44).

- The NAS committee raises the concern that "because Crump et al. (2008). argue that there are no data to refute these assumed and arbitrary adjustments of the Conolly et al. models, they state that the onus is on others to show that such small changes cannot occur (that is, prove a negative before the authors would accept the contention that the Conolly et al.

models are at all conservative as Conolly et al. suggested). That standard cannot be met" (p. 40).

Response: In a sensitivity analysis, one makes small changes to the inputs or assumptions in a model and observes the changes in the output. The purpose of such an analysis, as recommended by the cancer guidelines, is to establish that predictions from the BBDR model are robust. These changes should be small enough to be consistent with the data used to develop the model and biological constraints imposed on the model inputs and assumptions. EPA's sensitivity analyses presented in this assessment draft adhere rigorously to this requirement. In particular, in the context of model treatment of initiated cells (the focus of the above NAS comment) EPA's sensitivity analyses are based on extremely small variations to the initiated cell division rates assumed in the original model. These variations, as presented in the current assessment, are smaller by an order of magnitude than those carried out in Crump et al. (2008). The calculations were constrained to satisfy the conditions (as in as in Conolly et al., 2004) that model predictions provide good fits to:(a) the formaldehyde combined bioassay tumor incidence data (Monticello et al., 1996; Kerns et al., 1983) and (b) the background rates of respiratory cancers in humans obtained from the SEER database.

Furthermore, it was ascertained that the ratio of initiated cell division rate to initiated cell death rate was very close to the value of one for any variations in parameter values in the sensitivity analyses. For the variations presented in the current assessment, this ranged from 0.96 to 1.10, very similar to the range of 0.96 to 1.07 in Conolly et al. (2004).

There are no empirical data on division rates for these initiated cells; thus, these values were assumed in the original model. Therefore, in order to provide perspective on the variations in the division rates of initiated cells that were used for the purpose of the sensitivity analysis, the current assessment compares them with the empirical variability in normal cell division rates. These issues are addressed in the "biologically based dose response modeling" subsection of 2.2.1. EPA believes the sensitivity analysis variations in this assessment are consistent with the available data and biological constraints.

- In particular, adjustments of parameter values associated with mutation, birth, and death rates of initiated cells used in EPA's analysis of alternative models that yielded the most extreme deviations from the Conolly et al. (2004) low-dose extrapolations also produced unrealistically high added risks for humans at concentrations that have been observed in the environment of occupationally exposed workers (100% incidence at concentrations as low as about 0.1–1 ppm). Thus, the committee recommends that manipulations of model parameters that yield results that are biologically implausible or inconsistent with the available data be discarded and not used as a basis for rejecting the overall model (p. 42).

Response: The current assessment provides more refined sensitivity analyses (see "biologically based dose response modeling" subsection of 2.2.1). This includes specific comparisons of values for lifetime human MLE risk estimates between the values resulting from: 1) EPA's analysis of epidemiological data on nasopharyngeal cancers (NPC) from the National Cancer Institute (NCI) cohort study of workers occupationally exposed to formaldehyde, 2) the original Conolly et al. (2004) model for squamous cell carcinoma in humans as extrapolated from the F344 rat bioassays, and 3) EPA's sensitivity analyses of that model. The sensitivity analyses in the assessment shows that the original model and its variants, arising from extremely small variations in values of the unknown initiated cell

replication rates used in the original model, result in values that range from being many orders of magnitude different from, to substantially in agreement with, the lifetime risks projected from the epidemiology data. These model variations all adhere to the same biological constraints and provide similar fits to the tumor incidence data when used in the rat SCC model.

- In contrast, Conolly et al. (2003) focused their model parameter estimates to represent "best-fit," using maximum likelihood estimates, whereas Subramaniam et al. (2007) and Crump et al. (2008) pushed parameter assumptions in a single direction to show that different assumptions that fit the experimental data can yield different results of low-dose extrapolation (p. 43).

- Conolly and co-workers (Conolly et al., 2003) felt that they made several conservative assumptions in their models—use of hockey-stick rather than J-shaped models for cell proliferation, use of overall respiratory tract cancer incidence in humans to calculate basal mutation rates, and use of an upper bound on the proportionality parameter relating DPX to mutation. EPA pushed that concept further by making even more conservative assumptions within the models that cumulatively resulted in radical departures from the results of the Conolly et al. models with regard to low-dose extrapolation of tumor incidence. The committee notes that EPA forced changes in the model parameter values in a direction that yielded more conservative results rather than one that yielded a best fit to the data (p. 43).

  Response: EPA considered central estimates of input parameters. As the NAS supported in the comment above, the current assessment also appropriately examines uncertainties in the inputs and the sensitivity of modelling results to assumptions. For some modeling assumptions, there is no specific data from which to select a central estimate or maximum likelihood and EPA evaluates whether the model is sensitive to the assumptions and plausible alternatives. EPA's analysis evaluates a continuous range of minor perturbations to the original formaldehyde model that are all equally consistent with the data used in developing the model. Resulting risk estimates are both above and below (i.e., vary in both directions from) that obtained in Conolly et al. (2004). The risk estimates from some of the model implementations in the current draft are obtained without making conservative assumptions or calculating an upper bound; all these models retained the J shape for the dose response for normal and initiated cell replication. EPA's sensitivity analysis does not necessarily yield conservative results; risk estimates substantially below background levels of human risk are obtained from some variations in the division rates for initiated cells that are used in the sensitivity analyses. Thus, the analyses are not constrained to push the model output in a single direction.

- The committee was also struck by the relative lack of transparency in the draft IRIS assessment's description of the decision to use the peer-reviewed BBDR models minimally (p. 43).

- As a result of the agency's reanalysis of the models, EPA chose not to use the full rat and human BBDR models to estimate unit risks. Instead, in a benchmark-dose approach, EPA used the CFD-derived determinations of formaldehyde flux to the entire surface of mucus-coated epithelium to derive a point of departure based on nasal cancers in rats. It then extrapolated to zero dose by using a default linearized multistage approach. The committee is concerned about that approach for low-dose extrapolation. The committee found that the

evaluations of the original models and EPA's reanalysis conflicted with respect to the intent or purpose of using the formaldehyde BBDR models in human health assessments (p. 43).

- The primary purposes of a BBDR model are to predict as accurately as possible a response to a given exposure, to provide a rational framework for extrapolations outside the range of experimental data (that is, across doses, species, and exposure routes), and to assess the effect of variability and uncertainty on model parameters (p. 5).

- Given that the BBDR model for formaldehyde is one of the best-developed BBDR models to date, the positive attributes of BBDR models generally, and the limitations of the human data, the committee recommends that EPA use the BBDR model for formaldehyde in its cancer assessment, compare the results with those described in the draft assessment, and discuss the strengths and weaknesses of each approach (p. 5).

- A biologically based dose-response (BBDR) model that has been developed for formaldehyde could be used in the derivation of the unit risk estimates. EPA explored the uncertainties associated with the model and sensitivities of various model components to changes in key parameters and assumptions and, on the basis of those extrapolations, decided not to use the BBDR model in its assessment (p. 5).

Response: The current draft has improved transparency in regard to its use of the BBDR model and its evaluation for low-dose extrapolation. Because the BBDR modeling integrates various mechanistic information and time-to-tumor data from individual animals in the tumor bioassay, it improves the dose-response modeling of the observed nasal cancers in the F344 rat. EPA's current assessment uses two formulations of the BBDR model to estimate points of departure from the animal nasal cancer data, and to illustrate the uncertainties that arise in using these and other models for low-dose risk estimation. The BBDR modeling incorporates a precursor response in the form of labeling index data. This allowed EPA to evaluate PODs for nasal cancer risk at the 0.5% level (slightly below the range of the observed data) which is just below the dose where a change in the curvature of the dose response occurs. These PODs are based on formaldehyde flux to the tissue as a dose-metric calculated from fluid dynamic modeling of airflow and formaldehyde uptake in anatomically realistic representations of the upper respiratory tract. Extrapolation of these values to the human is also based on formaldehyde flux to the tissue using fluid dynamic modeling. Computational fluid dynamic modeling of formaldehyde flux to the nasal lining, is also used in deriving a candidate reference dose for squamous metaplasia observed in F344 rats.

However, EPA's analyses show that the human extrapolation modeling in Conolly et al. (2004) is numerically unstable on two accounts. It does not provide robust measures of human nasal SCC risk at any exposure concentration, and no particular value can be selected because of the extreme uncertainty. Therefore, this human model is not used for extrapolating to human environmental exposures from the observed tumor incidence in the rat. The current assessment also explains why its preferred estimates of human nasal cancer risks from formaldehyde are derived from the human epidemiology data rather than from extrapolations of the animal study data.

As recommended by the NAS, the current assessment contrasts lifetime human risk estimates for cancer in the human respiratory tract from the formaldehyde BBDR model with other estimates in Section 2.2 of the toxicological review.

- The committee is also concerned that EPA directed substantial effort toward refuting many of the assumptions and conclusions of the Conolly et al. (2004, 2003) models rather than trying to fill the data gaps that were clearly articulated by the models. Conolly and co-workers were clear on that point and expressed the need for new data that could anchor many of the parameter values that had to be optimized from rather sparse data sets (p. 44).

  Response: EPA agrees that the formaldehyde BBDR model has helped identify data gaps. A large data gap identified by EPA is information on division rates of initiated cells in the respiratory tract. As suggested by the NAS such information can be used to anchor uncertain parameter values. Similar efforts have been directed in the area of modeling liver cancers to inform the health risk assessments for dioxin and other chemicals. In those cases, data on foci or nodules[35] have been used to estimate rates of initiation and proliferation, under the assumption that they are preneoplastic lesions. However, such foci or nodules have not been identified in the case of nasal cancer. As acknowledged by the NAS, assuming that initiated cells related to tumors in the respiratory tract can be identified, measurement of their division rates would be extremely difficult. Even if this difficulty were to be surmounted, it is reasonable to suppose that these rates would be at least as variable as division rates of normal cells. Based on the normal variation in such rates observed in normal cells, and the extreme sensitivity of the formaldehyde model to small differences in assumed division rates of initiated cells, EPA concludes that it would be impossible to measure these accurately enough to restrict the range of risks consistent with the model sufficiently to be useful for practical risk assessment needs. In the case of preneoplastic foci in the liver, it has not been possible to confidently decide which cells in foci or nodules represent initiated cells or even whether the model formulation is correct for those foci (Kopp-Schneider et al., 1998). Quantitative estimates of risk can be very sensitive to these choices.

- EPA's rationale for use of a low-dose linear extrapolation (through zero dose) is the observed linear relationship between DPX and exposure. The committee evaluated the strength of this rationale on the basis of [differences in] model predictions in Conolly et al. (2003) and Subramaniam et al. (2007) for the value of the constant of proportionality relating DPX to the probability of mutation in the BBDR modeling. However, the committee had low confidence in deciding which of these approaches was the most scientifically defensible because too few parameters were experimentally fixed and too many optimized against one data set [in either case].

- The current parameter estimates that Conolly et al. (2003) optimized from the data, using a maximum likelihood function, suggest that the proportionality constant for DPX adding to the mutation rate of a normal (or intermediate) cell should be zero or close to zero. That suggests that DPX is not directly related to the key events leading to mutation and carcinogenicity per se. Because this [i.e., mutagenic potential being proportional to DPX burden] is the only low-dose linear relationship between exposure and a biomarker of response, EPA contends that the low-dose extrapolations should be linear through zero dose. For example, Subramaniam et al. (2007) examined alternative choices to parameters associated with DPX clearance and suggested that in the exposures at which tumors were seen, the mutagenic mode of action could contribute up to 74% of the added tumor probability. Because too few parameters were experimentally fixed and too many

[35]To our knowledge, no such preneoplastic foci have been seen for squamous cell carcinomas.

optimized against one data set, confidence in deciding whether the Conolly et al. or the Subramaniam et al. approach is the most scientifically defensible is not high (p. 39).

Response: EPA is assuming that the NAS comment on low-dose extrapolation refers to extrapolating the risk of nasal tumors from the rat to human. We agree with the committee's conclusion that neither the Subramaniam et al. (2007) nor the Conolly et al. (2004) analyses should be used as the basis for making a mode of action determination. EPA's decision to use a linear extrapolation to the origin from a point of departure was based only on the following two considerations: (1) that the BBDR models did not constrain estimates of human respiratory cancer risk at any exposure concentration, and did not constrain estimates of rat nasal cancer risk at exposure concentrations below the observed data in the rat and (2) EPA's determination, based on multiple sources of data in humans and animals, of a mutagenic contribution to formaldehyde's carcinogenic potential in the upper respiratory tract of exposed humans.

Subramaniam et al. (2007) did not attempt to determine the most appropriate low-dose relationship. Rather, their analysis, and the use of their results in the current assessment, expresses the uncertainty in the assertion in Conolly et al. (2004) that formaldehyde's mutagenicity, as per their model conclusions, did not play a role in its carcinogenicity. The current assessment further clarifies this point of view.

- The reanalysis by Subramaniam et al. (2007) is used to support the mutagenic mode of action of formaldehyde and to reduce support for using the BBDR models on the basis of the uncertainties in parameter estimation and assumptions in the models (p. 43).

Response: The determination that formaldehyde's direct mutagenic action contributes to its carcinogenicity in humans was based on multiple sources of data in humans and laboratory animals. These are detailed in Section 1.2.5 of the assessment. The analyses in Subramaniam et al. (2007) and in other BBDR model implementations pursued in the current assessment were partly used to evaluate the uncertainty in an inference on mode of action made by Conolly et al. (2004). Specifically, based on BBDR modeling results, these authors inferred that formaldehyde's mutagenicity did not play a role in its carcinogenicity. EPA's uncertainty analyses of the BBDR modeling determined that such an inference was extremely uncertain. To be clear, in some alternate model implementations EPA estimated parameter values that were consistent with a significant role for formaldehyde's putative mutagenic action in explaining its tumorigenicity, but these results were not the basis upon which EPA concluded that there was sufficient weight of evidence for a mutagenic MOA for upper respiratory tract cancers. The currentassessment makes this very clear.

- Because multiple modes of action may be operational, the committee recommends that EPA provide additional calculations that factor in regenerative cellular proliferation as a mode of action, compare the results with those presented in the draft assessment, and assess the strengths and weaknesses of each approach. (pp. 5) Although the draft IRIS assessment discusses that [regenerative cell proliferation associated with cytotoxicity] mode of action, it relies on the mutagenic mode of action to justify low-dose extrapolations. The committee recommends that EPA provide alternative calculations that factor in nonlinearities associated with the cytotoxicity compensatory cell proliferation mode of action and assess the strengths and weaknesses of each approach (p.44).

Response: Because multiple modes of action are operational, EPA's assessment uses BBDR modeling that factors in the empirical regenerative cellular proliferation data, thus, inherently including the nonlinearity to which the above comment points, as well as the DNA protein cross-link data representing formaldehyde's directly mutagenic potential. The cancer slope factors derived in the assessment from the animal nasal cancer data are consistent with the predictions of the BBDR modeling. The current assessment also compares with the $BMDL_{01}$ derived exclusively from regenerative cell proliferation by Schlosser et al. (2003). These authors fitted a curve with a threshold in dose to the exposure time-weighted average (over the entire nose) of the unit length labeling index data from Monticello et al. (1996; 1991). While these points of departure are in agreement with each other, the BBDR modeling points to significant risk below the presumed threshold in Schlosser et al.

The current assessment also notes that, because the BBDR modeling estimates the constant of proportionality relating DPX levels to formaldehyde-induced mutation by fitting to the steeply rising tumor incidence data, EPA's uncertainty analysis of results derived from the modeling reflects [model] uncertainty associated with a putative mutagenic mode of action.

- The committee agrees with EPA that existing data are insufficient to establish the potential biologic variability in model parameters associated with the mutagenic mode of action adequately. However, because the mutagenic mode of action is the major reason for adopting the default low-dose linear extrapolation methods over application of the BBDR models in the draft assessment, the committee recommends that the manipulations that lead to such high contributions of mutagenicity to the mode of action for nasal tumors be reconciled with the observations that formaldehyde is endogenous, that nasal tumors are very rare in both rats and humans, and that no increases in tumor frequency have been observed in animal studies at formaldehyde exposure concentrations that do not also cause cytotoxicity (p. 42).

Response: EPA agrees with the NAS that there are no data to directly establish the variability or uncertainty in key unknown model parameters. The EPA cancer guidelines note that unless there is an established mode of action known to be inconsistent with a linear estimate of upper-bound risk at low doses, it is EPA's practice to use a linear approach to estimating an upper-bound on the low-dose risk. That cancers may be due to a mutagenic mode of action is one rationale for that policy. But, dose-response functions for a human population may also be approximately linear at low doses due to other factors, including the effect of variation in human responses, as was noted in the NAS report on Science and Decisions (NRC, 2009). It is noted that the assessment addresses the extra risk associated with inhaled formaldehyde and is not providing estimates of the risk that might be associated with the endogenous formaldehyde concentration.

EPA has examined the range of risk estimates obtained when using the BBDR modeling approach in Conolly et al. (2004) for extrapolation in a manner that reflects uncertainty and variability. This approach is not constrained to assuming a mutagenic mode of action, and incorporates data related to formaldehyde mutagenicity as well as formaldehyde's effect on cell proliferation. This course of action follows NAS advice. As explained earlier, the range in risk estimates resulting from the BBDR modeling is so large that low-dose risk cannot be constrained in either the rat or the human. Thus, given the uncertainty, it is reasonable to use a linear extrapolation from a point of departure estimated using the BBDR modeling (and more than one point of departure was determined to reflect model uncertainty). EPA

also verified that linear extrapolation is not inconsistent with the large range of risk estimates predicted if the BBDR modeling were to be used below the POD.

It is important to note that the model predicts extra risk (over background risk) due to inhaled exogenous concentrations of formaldehyde. EPA's uncertainty analyses with the rat formaldehyde BBDR model include the observation of tumors in historical control animals from NTP inhalation bioassays. Therefore, these model implementations were calibrated to predict the observed levels of spontaneous tumor incidence. Thus, these predictions are presumably consistent with contributions to baseline risk [if any] arising from endogenous levels of formaldehyde. The rarity of squamous cell carcinoma in rats is appropriately accounted for by the inclusion of historical control animals from inhalation bioassays. The alternate model implementations and the perturbations considered in initiated cell replication rates were all constrained to reproduce the tumor incidence data. Specifically, model fits to the time-to-tumor data in all cases were equivalent. In other words, all these results were consistent with no increases in observed tumor frequency in animal studies at subcytotoxic formaldehyde exposure concentrations.

- Crump et al. (2008) made an arbitrary change in the DPX-based effect on initiated cell replication by theorizing that if an initiated cell is created by a specific mutation that impairs cell-cycle control, there may be a mitigation of cell replication that is observed in the low-dose cell proliferation of normal cells (that is, in the negative vs baseline replication portion of the J-shaped dose-response curve) and hence a shift of the cell division of an initiated cell in the model toward greater rates at low doses (p. 40).

- The change disconnects the birth and death rates of initiated cells from constraints used by Conolly et al. (2004) based on normal cells. The committee concludes that this change is contrary to the explanation provided by Monticello et al. (1996), who suggested that it is not a mutation in cell-cycle check points that results in lower cell-division rates than control at low exposures but rather an increase in the time that it takes for DNA-repair processes to eliminate the DPX before the cell can resume the process of cell division that leads to lower than basal cell-division rates at low exposures. These are two fundamentally different mechanisms with different connotations for risk—the mutagenic one chosen by EPA and the DNA-repair mode of action supported by several other publications on DPX cited by Conolly et al. (2004, 2003) and Monticello et al. (1996) (p. 40).

Response: The current assessment does not rely upon the mechanistic hypothesis put forward in Crump et al. (2008) for what might cause cell-division rates to be lower than control at low exposures. (EPA has removed speculation as to how minor differences between initiated and other cells could arise.) Nonetheless, any mechanistic arguments that one might advance for a J-shaped curve for a dose-response relationship for cell replication should equally apply to the perturbations made for the sensitivity analyses. The current assessment explains that small potential differences in the division rates of initiated cells examined in the sensitivity analysis are illustrative that, as the NAS comment notes, the biological data are not available to directly determine whether initiated cells have the same or different division rates as uninitiated cells. The perturbations considered in the sensitivity analyses in the current draft EPA assessment are substantially smaller than in Crump et al. (2008) and are only applied to the J-shaped dose response for cell replication in the original model. The sensitivity analysis also adheres to the constraint used in Conolly et al. (2004) that the growth advantage of initiated cells over normal cells is kept close to

1.0. For the variations presented in the current assessment, this ranged from 0.96 to 1.10, very similar to the range of 0.96 to 1.07 in Conolly et al. (2004).

- There were zero squamous cell carcinomas in control rats in the two bioassays used to define the basal mutation rates of normal and intermediate cells in the two-stage, MVK dose-response model. Conolly et al. (2004) used results from the full National Toxicology Program historical control database. That is a point of contention by EPA, which believes that only historical controls from inhalation bioassays (and those in the same laboratory as the formaldehyde study) can be used in a relevant comparison. Squamous cell carcinomas are so rare that some leeway in approximating basal rates may have to be accepted, even though EPA's point is technically correct (p. 40).

  Response: EPA agrees. The rarity of squamous cell carcinoma in rats is appropriately accounted for by the inclusion of historical control animals from inhalation bioassays in EPA's uncertainty analyses. Given the reactivity of formaldehyde, to allow for a reasonable comparison it is considered essential that studies used the same route of exposure; as such, noninhalation studies were not included in the current analyses.

- Estimating parameters for basal mutation rates for a normal to intermediate and intermediate to malignant transformation in humans is subject to even more uncertainty than in the rat.

  Response: EPA agrees and has included this in additional uncertainties associated with the formaldehyde human model.

- The first-order clearance of DPX could be slower than that used by Conolly et al. (2004, 2003). Over time, epithelial tissue in targeted regions of the nose thickens. The thickening could conceivably dilute DPX concentrations in the measured tissues to such an extent that residual concentrations 18 hr after exposure are not different from those in naïve animals, and this would affect the determination of DPX clearance rates (pp. 41).

  Response: The current assessment discusses the uncertainty in clearance rates of DPX and its impact on model calibration.

Health endpoints

- Overall, the committee found that the noted outcomes were appropriate to evaluate. EPA identified relevant studies for its assessment, and on the basis of the committee's familiarity with the scientific literature, it does not appear to have overlooked any important study. For a few outcomes, however, as noted below, EPA did not discuss or evaluate literature on mode of action that could have supported its conclusions. Although EPA adequately described the studies, critical evaluations of the strengths and weaknesses of the studies were generally deficient, and clear rationales for many conclusions were not provided. In several cases, the committee would not have advanced a particular study or would have advanced other studies to calculate the candidate RfCs (p. 6).

  Irritation

- The committee notes that EPA did not (but should) review research findings on transient-receptor-potential ion channels and evaluate the use of this evidence for improving

understanding of the mode of action for sensory irritation and respiratory effects attributed to formaldehyde exposure (p. 6; and list at end of Chapter P 52).

Response: EPA agrees with this recommendation and discusses involvement of transient-receptor-potential ion channels in a more comprehensive MOA discussion for noncancer respiratory tract-related effects, including sensory irritation (see Section 1.2.1).

- Although the chamber studies are of acute duration, they are complementary with the residential studies and provide controlled measures of exposure and response. Therefore, the committee recommends that EPA present the concentration response data from the occupational, chamber, and residential studies on the same graph and include the point estimate and measures of variability in the exposure concentrations and responses (p. 6; also in list at end of the chapter, pp. 52–53).

Response: EPA agrees with this recommendation and presents the dose-response results from the literature in graphical form. The prevalence of eye irritation (and standard errors) reported by the studies of residential populations and controlled human exposure studies are plotted on the same graph in the range of formaldehyde concentrations that are common to both (0–1 mg/m^3). Because the controlled human exposure studies examined symptoms at higher concentrations as well, an additional graph that includes all of the data also is included. The results of the occupational studies on irritation symptoms are complementary, but the variation in exposure levels in the exposed groups in these settings was large, and trends with exposure generally were not described. These data were less informative compared to the exposure-response information from the residential or controlled human exposure studies.

- The committee found that EPA dismissed the results of the exposure chamber and other nonresidential studies too readily. Although the exposure durations for the chamber studies are short relative to the chronic duration of the RfC, the studies provide complimentary information that could be used for deriving a candidate RfC (also in list at end of the chapter on p. 52).

Response: EPA agrees that the controlled human exposure studies provide complementary information and integrated this evidence in concert with those of the occupational and residential studies. In accordance with the criteria for selecting studies for the derivation of candidate RfCs (see Section 2.1.1), EPA uses the dose-response information from epidemiology studies of residential exposure because studies of good quality are available (Liu et al., 1991; Hanrahan et al., 1984) and compares these to cRfCs derived from medium confidence controlled human exposure studies (Kulle, 1993; Andersen and Molhave, 1983).

- The committee agrees with EPA's selection of eye irritation as a critical sensory-irritation effect caused by formaldehyde exposure because residential, occupational, and chamber studies have demonstrated that the eyes are more sensitive to irritation from formaldehyde than the nose and throat.

Response: EPA agrees that irritant effects on the eye are a sensitive response to formaldehyde.

- The committee supports EPA's advancement of the residential studies by Liu et al. (1991) and Hanrahan et al. (1984) for derivation of candidate RfCs as adequately conducted studies

of a randomly selected general population and agrees with the points of departure identified by EPA from these studies:

LOAEL = 95 ppb (Liu et al., 1991)

BMCL10 = 70 ppb (Hanrahan et al., 1984)

Response: EPA's rationale for selecting study results for the derivation of candidate RfCs is provided in the current draft. These two studies are included among those for which candidate RfCs were considered. Although the results from Liu et al. (1991) were not used to derive a cRfC, the data can be used to check the estimated POD based on Hanrahan et al. (1984).

- Chapter 4: The committee recommends that EPA address the following in the revision of the formaldehyde draft IRIS assessment.

- Strengthen its critical evaluation of the studies.

- **Response:** In the current draft assessment, studies identified as meeting the PECO criteria were evaluated for their ability to inform the hazard reviews using standardized approaches and were categorized by a level of confidence (*high*, *medium*, *low*, and *not informative*). The issues pertinent to evaluating the strengths and limitations of individual studies with respect to specific health endpoints are discussed, and each study evaluation is documented in tables found in the supplemental material for each health hazard (Appendix A.5). The results of the study evaluations (e.g., confidence) are included in the evidence tables and figures that summarize the studies found in each hazard section of the toxicological review. Not advance the Ritchie and Lehnen (1987) study for calculation of a candidate RfC.

Response: EPA agrees with this recommendation and does not advance Ritchie and Lehnen (Ritchie and Lehnen, 1987) to derive a candidate RfC.

Decreased pulmonary function

- The committee agrees with EPA that formaldehyde exposure may cause a decrease in pulmonary function, but EPA should provide a clear rationale to support that conclusion (p. 6).

Response: In the current assessment, the studies of pulmonary function were evaluated and synthesized using a common framework applied to all hazard categories and outcomes. The studies are described in tables categorized according to confidence in the study results determined by systematic evaluation of risk of bias and sensitivity. The study evaluations, with the strengths and limitations of the studies, are documented in supplemental material (see Appendix A.5.3). The evidence integration section provides the summary rationale supporting the hazard judgment.

- Furthermore, although the committee supports the use of the study by Krzyzanowski et al. (1990) to calculate a candidate RfC, EPA should provide a clear description of how the study was used to estimate a point of departure and should also consider the studies conducted

by Kriebel et al. (2001; 1993), and the chamber studies for possible derivation of candidate RfCs (p. 6; also at end of the chapter).

Response: The description of how the POD for Krzyzanowski et al. (1990) was derived is described (see Section 2.1 of the toxicological review and Appendix B.1.2). EPA evaluated study results from Kriebel et al. (2001; 1993) to develop a candidate RfC and decisions for the selection of studies to derive a cRfC are documented. Kriebel et al. (2001) is described in the toxicological review (Section 1.2.2). Estimation of a cRfC using these data is not straightforward due to the simultaneous modeling of the two exposure estimates and the complication of potential covariance between these effects. Therefore, a POD could not be determined from these data. The controlled human exposure studies of pulmonary function were not included in the evaluation of the hazards of subchronic or chronic exposures because these studies exposed subjects only for minutes or hours while the review focused on effects related to exposure over a prolonged period.

- The committee recommends that EPA address the following in the revision of the formaldehyde draft IRIS assessment:

- Prepare plots of the findings of the chamber studies to assess the use of pooling their results.

Response: The controlled human exposure studies of pulmonary function were not included in the evaluation of hazard because these studies exposed subjects only for minutes or hours to high concentrations while the review focused on effects related to exposure over a prolonged period. Several studies more relevant to the long-term exposure setting that was the focus of this review were available.

- Provide further justification for its choice of the study by Krzyzanowski et al. (1990) for estimating the point of departure.

Response: The current draft assessment contains a detailed discussion and rationale for why the study by Krzyzanowski et al. (1990) was selected for the development of a candidate RfC (see Section 2.1.1).

Respiratory tract pathology

- Animal studies in mice, rats, and nonhuman primates clearly show that inhaled formaldehyde at 2 ppm or greater causes cytotoxicity that increases epithelial-cell proliferation and that after prolonged inhalation can lead to nasal tumors. Although the committee agrees with EPA that the human studies that assessed upper respiratory tract pathology were insufficient to derive a candidate RfC, it disagrees with EPA's decision not to use the animal data (pp. 6–7).

Response: EPA agrees with this point and has evaluated the toxicology studies reporting respiratory tract pathology to identify a POD and derive a candidate RfC based on incidence of squamous metaplasia (Woutersen et al., 1989; Kerns et al., 1983) (see Section 2.1.1).

- The committee concludes that a candidate RfC should be calculated for noncancer pathology of the respiratory tract (that is, in the nasal epithelium).

Response: EPA agrees with this point and has evaluated the studies reporting respiratory tract pathology to identify a POD and derive a candidate RfC based on incidence of squamous metaplasia (Woutersen et al., 1989; Kerns et al., 1983) (see Section 2.1.1).

- Do not calculate a candidate RfC for mucociliary clearance.

Response: EPA has not calculated a candidate RfC for mucociliary clearance.

Asthma

- In infants and children, wheezing illnesses that are the result of lower respiratory tract infections are often labeled as asthma, and in adults, the symptoms can overlap with those of other chronic diseases, such as chronic obstructive pulmonary disease. Thus, a critical review of the literature is essential to ensure that what is being evaluated is asthma. The committee notes that this issue is not adequately addressed in the draft IRIS assessment and that EPA advanced a study (Rumchev et al., 2002) that most likely suffers from misclassification of infection-associated wheezing in young children as asthma (pp. 7 and 61).

Response: EPA agrees that the condition experienced by the children in the Rumchev et al. (2002) study is unlikely to represent the asthma phenotype that characterizes the majority of research in childhood asthma (with onset typically in grade school). EPA developed criteria to evaluate the definitions for the measures of allergy, asthma and other respiratory outcomes reported in the epidemiology studies. This process included consultations with two groups of clinical and epidemiology experts in allergy and asthma regarding the reliability, validity, and interpretation of various types of outcome measures used in the identified observational epidemiology studies. Based on these criteria, the study by Rumchev et al. (2002) is not included in the set of studies examining asthma.

- The draft IRIS assessment also provides little discussion of the current understanding of the mechanisms of asthma causation and exacerbation. Given the abundant research available, the committee recommends that EPA strengthen its discussion of asthma to reflect current understanding of this complex disease and its pathogenesis (pp. 7).

- Asthma is a complex phenotype on whose pathogenesis substantial research has been conducted. The discussion of asthma needs to be strengthened to reflect the extensive literature better. The discussion of mode of action needs to be greatly strengthened and grounded in current understanding of pathogenesis. The current speculative discussion is not satisfactory (p. 61).

RESPONSE: EPA agrees with these two suggestions. The pathogenesis of asthma, as currently understood, and the potential mode(s) of action through which formaldehyde may act in the exacerbation of this condition, are discussed in a more comprehensive MOA discussion for portal of entry noncancer effects, including asthma and immune-related endpoints (see Section 1.2.3 of the Toxicological Review).

- Although the committee agrees that the study by Garrett et al. (1999a) should be used to calculate a candidate RfC, the approach taken to identifying the point of departure needs further justification (p. 7).

RESPONSE: In the current draft assessment, the Garrett et al. (1999a) study was considered for the derivation of a candidate RfC for allergic sensitization, but was not advanced because of uncertainty with respect to the timing of the exposure measure and its relation to skin prick test results.

- The committee recommends that EPA address the following in the revision of the formaldehyde draft IRIS assessment: Strengthen the discussion of asthma to reflect current understanding of this complex phenotype and its pathogenesis better. There should be greater clarity regarding the outcomes considered: incident asthma (the occurrence of new cases), prevalent asthma (the presence of asthma at the time of study), or exacerbation of established asthma (p. 61).

Response: As indicated in response to previous comments, EPA agrees with this suggestion. Based on EPA's consultation with clinical and epidemiology asthma experts, EPA selected the definitions of disease that would be reviewed. These included incident asthma, studies of prevalence of current asthma (typically ascertained based on frequency of symptoms or medication use over the past 12 months), and studies of asthma severity or asthma control (frequency of symptoms or medication use over a short period of time, e.g., 2–4 weeks).

Respiratory tract cancer

- However, the draft IRIS assessment does not present a clear framework for causal determinations and presents several conflicting statements that need to be resolved regarding the evidence of a causal association between formaldehyde and respiratory tract cancers. On the basis of EPA cancer guidelines, the committee agrees that there is sufficient evidence (that is, the combined weight of epidemiologic findings, results of animal studies, and mechanistic data) of a causal association between formaldehyde and cancers of the nose, nasal cavity, and nasopharnyx. It disagrees that the evidence regarding other sites in the respiratory tract is sufficient (pp. 9 and 87).

- EPA's review of the literature on formaldehyde and respiratory cancer was thorough and appropriate. It would be useful if, in the future, EPA could explicitly state its criteria for evaluation of the evidence of causality based on its own cancer guidelines. Several sections of the draft IRIS assessment contain conflicting statements on the evidence of causality that clearly need to be rectified. The committee finds that, on the basis of EPA's guidelines, there is sufficient evidence of a causal association between formaldehyde and cancers of the nose and nasal cavity (ICD8 160) and nasopharynx (ICD8 147) but not other sites of respiratory tract cancer (p. 87).

Response: The epidemiological and toxicological studies of respiratory cancer were evaluated for risk of bias and sensitivity and were categorized according to the level of confidence (*high*, *medium*, and *low*) in the study results to inform the hazard assessment. The study results were synthesized, and the evidence integrated for each respiratory cancer category using the framework described in the Preface. The Preface of the Toxicological Review explicitly describes the criteria used to evaluate the evidence to draw conclusions in a manner consistent with the EPA cancer guidelines.

- The committee agrees that the study by Hauptmann et al. (2004b) is an appropriate choice for the derivation of a point of departure and unit risk. Although it is a high-quality study, it is important to recognize some of its deficiencies, such as the apparent inconsistency

between the findings in different plants in the study and the weakness of the exposure-response relationship in connection with cumulative exposure. Furthermore, the study was found to be missing deaths in a later update of the cohort for lymphatic and hematopoietic cancers. NCI is updating its cohort for respiratory cancer and other solid tumors. The update not only will include the missing deaths but will extend the follow-up, and this will result in nearly twice the amount of deaths (pp. 9 and 88).

Response: Consistent with the evaluation of all relevant studies considered in the toxicological review using standardized approaches, the cohort followed by the Hauptmann et al. (2004b) study was evaluated for risk of bias and sensitivity, and this evaluation is documented in the supplemental material (see Appendix A.5.9) and in the evaluation of hazard (see Section 1.2.5). EPA has incorporated the updated follow-up of this cohort (Beane Freeman et al., 2013) in its synthesis of the epidemiological studies and used these data in the derivation of the unit risk value.

Immunotoxicity

- The draft IRIS assessment presents numerous studies suggesting that formaldehyde has the ability to affect immune functions. However, EPA should conduct a more rigorous evaluation of the strengths and weaknesses of the studies; more integration of the human and animal data would lend support to the conclusions made. The committee agrees with EPA's decision not to calculate a candidate RfC on the basis of immunotoxicity studies (p. 10).

Response: The current draft includes a discussion of the quality of the studies of immune function using the approach developed for evaluating all epidemiology studies in the assessment. As both part of this review and to organize the hazard analysis, advice from allergy experts was incorporated concerning the interpretation of the allergy outcome measures evaluated in epidemiology studies. The hypersensitivity-relevant animal experimental studies provide mechanistic support and were integrated with the epidemiology studies in evaluating the weight of evidence for immune system hazard. Although the animal toxicology studies were not used to derive a candidate RfC, results from several epidemiology studies contributed to the development of candidate RfCs for allergy-related conditions and asthma.

- The committee agrees with EPA's decision not to calculate a candidate RfC for immunotoxicity at this time. The committee recommends, however, that EPA address the following in the revision of the formaldehyde draft IRIS assessment:

- Provide a more careful evaluation of the relative strengths and weaknesses of the key studies.

Response: In the current draft assessment, studies identified as meeting the PECO criteria were evaluated for their ability to inform the hazard reviews using standardized approaches and were categorized by a level of confidence (*high*, *medium*, *low*, and *not informative*). The issues pertinent to evaluating the strengths and limitations of individual studies with respect to specific health endpoints are discussed, and each study evaluation is documented in tables found in the supplemental material for each health hazard (Appendix A.5). The level of confidence in each result is included in the tabular displays and discussion of studies in the toxicological review.

- Consider giving additional weight to animal studies in which exposure assessment was more rigorously controlled (p. 97).

Response: Details of the exposure protocol, including level of control and source of formaldehyde, were explicitly considered in the evaluation of controlled exposure studies in animals, and was a driving factor in study confidence determinations (see Appendix A.5). However, due to limitations in the animal models used to evaluate hypersensitivity-related responses, these data were used to inform MOA analyses only (see Section 1.2.3).

Neurotoxicity

- The committee found that EPA overstated the evidence in concluding that formaldehyde is neurotoxic; the human data are insufficient, and the candidate animal studies deviate substantially from neurotoxicity-testing guidelines and common practice. Furthermore, the committee does not support EPA's conclusion that the behavioral changes observed in animals exposed to formaldehyde are not likely to be caused by the irritant properties of formaldehyde. Data indicate that those changes could occur as a result of nasal irritation or other local responses; stress, also an important confounder that can affect the nervous system, was not considered by EPA. The draft IRIS assessment provides conflicting statements that need to be resolved about whether formaldehyde is a direct neurotoxicant (p. 10).

Response: EPA has updated and reconsidered the existing body of evidence for neurotoxicity. The section in the current draft clearly presents the strengths and limitations of each study, as well as the relative contribution each study made to the overall conclusions related to potential nervous system effects of formaldehyde exposure.

Regarding the human data, the NRC indicated that the causal association between formaldehyde exposure and ALS in one study (Weisskopf et al., 2009) was overstated. Accordingly, a more detailed discussion of this study and its conclusions, as well as related studies that have been published since the NRC review, have been added to the current text. A candidate RfC is no longer derived. As in the previous draft, the co-exposure limitations of the Kilburn et al. studies are acknowledged and discussed. In the current assessment, the data from controlled human exposure studies are now evaluated in greater detail.

In the current draft, endpoints in animal studies are critically evaluated alongside the human data. The candidate animal studies relying on open field testing endpoints are no longer considered for developing candidate values. In addition, the discussion of nonguideline test paradigms, including the specific behavioral correlates they may be capable of distinguishing, has been expanded in the text. The rodent-specific irritant response, reflex bradypnea, is now explicitly considered for each study relevant to interpreting the potential neurotoxicity hazard (see Appendix A.5.7). In addition, discussion of behaviors evaluated at formaldehyde levels at which irritant-related processes in rodents are expected has been added, and endpoints which are clearly reliant on olfaction-related behaviors [e.g., odor-cued conditioning in (Sorg and Hochstatter, 1999)], in particular, are considered likely to be influenced by irritation and studies that also examined the potential for nasal damage were preferred. The current draft includes a more rigorous examination of the formaldehyde inhalation exposure methods used across studies, which is now a critical consideration for evaluating how well individual studies inform the potential for formaldehyde-induced neurotoxicity. When contamination with

methanol was identified, or when the test article was not reported, the studies are now attributed much less weight in the overall database and discussions of possible confounding by methanol-induced toxicity have been added to the current text.

Potential stress-induced changes by formaldehyde, which can complicate the interpretation of other behaviors, are themselves considered to be highly relevant effects of exposure. This is now more fully discussed. Additionally, the current draft now considers the potential for contributions from stress or other uncontrolled variables to the observed behavioral responses. Unfortunately, the design of many of the identified studies does not permit a separate evaluation of immediate, stress-induced behaviors and possible direct effects of formaldehyde on neurobehavior. Stress-related changes that persist after exposures are terminated (e.g., neural sensitization; altered habituation) are now interpreted with greater concern.

EPA agrees that the lack of systemic availability of formaldehyde and its metabolites makes it highly unlikely that inhaled formaldehyde is a direct neurotoxicant. This viewpoint is now presented throughout the document (it is now an underlying assumption), and only potential mechanisms for indirect actions of inhaled formaldehyde arediscussed. As stated in the U.S. EPA *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), indirect effects of exposure are still considered to provide evidence of neurotoxicity.

Evidence of neurotoxicity at exposure levels comparable to respiratory system effects has not been conclusively shown for any neurotoxicity endpoint; this is clearly presented in the current draft. EPA agrees that nearly all of the controlled exposure studies, including the animal neuroanatomical changes, have significant limitations that reduce their ability to inform the hazard assessment. The limitations of these studies (including lack of clear exposure-response relationships, study design deficiencies, possible confounders, and a lack of database corroboration for specific endpoints) has been more transparently described in the text (see Section 1.3.1 of the Toxicological Review). Overall, the current evidence on neurotoxicity is considered insufficient to support causality in the current draft.

- The committee concludes that the draft IRIS assessment overstates the evidence that formaldehyde is neurotoxic. The selected studies are not sufficiently robust in design to be considered well executed for the purpose of neurotoxicity-hazard identification. One study of rats by Malek et al. (2003a) was advanced by EPA for consideration. It was considered to offer information on an outcome relevant to humans at an appropriate concentration. Appropriately, the study was not used to calculate a candidate RfC, partly because of uncertainty in extrapolating from the exposure conditions in the study to a chronic-exposure scenario (pp. 101–102).

Response: The current draft thoroughly reviews the existing body of evidence for neurotoxicity andmore clearly delineates the significant shortcomings of the available studies. However, while limitations in the methodology of the available studies precludes identification of a hazard, this is seen as an area of concern deserving further research.

Detailed discussions of study limitations have been added to the document text, based on thorough evaluations of the testing methodology and validity for each assessed endpoint (see Appendix A.5.7). The study by Malek et al. (2003a) is not advanced for consideration in the current draft.

- The committee agrees with EPA's decision not to calculate a candidate RfC on the basis of the neurotoxicity studies (p. 10).

Response: EPA agrees with the committee's recommendation and, in the current draft, EPA does not calculate a candidate RfC on the basis of the neurotoxicity studies.

- The committee recommends that EPA address the following in the revision of the formaldehyde draft IRIS assessment:

- Reevaluate its conclusions that behavioral changes are unlikely to be related to irritant properties of formaldehyde (p. 102).

Response: EPA agrees that irritation-related behaviors can have a significant influence on many of the neurobehavioral changes observed following formaldehyde inhalation. A more detailed consideration of the latency between exposure and testing, as well as the formaldehyde concentrations assessed, is now included in evaluations of individual studies (see Appendix A.5.7) and in the synthesis text as discussion points related to confounding. However, although it has not been sufficiently tested, an additional discussion has been added regarding the potential for repeated formaldehyde-induced irritation to elicit indirect, persistent neurological effects.

- Resolve inconsistencies regarding the concentration at which systemic effects of formaldehyde exposure are expected. The draft IRIS assessment indicates that there is some question as to whether formaldehyde should be considered a direct neurotoxicant, and some portions of the assessment suggest that systemic effects are unexpected at formaldehyde concentrations less than 20 ppm. That statement is inconsistently made in other parts of the document (p. 102).

Responses: EPA agrees that the previous draft contained inconsistent statements regarding direct or indirect neurological effects of formaldehyde. The current assessment does not include any text identifying formaldehyde as a direct neurotoxicant. The available neurotoxicity studies are insufficient to draw conclusions as to what formaldehyde concentrations might be expected to elicit systemic, nervous system effects. In the animal studies, the suggestive evidence of indirect neurotoxicity, defined in accordance with the neurotoxicity guidelines, is generally reported at formaldehyde concentrations well above observations of direct toxicity in portal-of-entry systems. Potential mechanisms for indirect neurotoxicity are now succinctly stated in the hazard synthesis, with an emphasis on their highly speculative nature.

Reproductive and developmental toxicity

- The draft IRIS assessment states that epidemiologic studies provide evidence of a "convincing relationship between occupational exposure to formaldehyde and adverse reproductive outcomes in women." The committee disagrees and concludes that a small number of studies indicate a suggestive pattern of association rather than a "convincing relationship" (p. 10).

Response: The epidemiological and toxicological studies of reproductive and developmental effects were evaluated for risk of bias and sensitivity (see Appendix A.5.8) and were categorized according to the level of confidence (*high*, *medium*, and *low*) in the

study results to inform the hazard assessment. The study results were synthesized and the evidence integrated for each outcome category using the framework described in the Preface. Regarding "adverse reproductive outcomes in women," using this evidence integration framework, EPA concluded that the **evidence indicates** that inhalation of formaldehyde likely causes increased risk of developmental or female reproductive toxicity in humans based on *moderate* evidence in observational studies finding increases in TTP and spontaneous abortion risk among women exposed to occupational formaldehyde levels. The pertinent evidence in animals is *indeterminate*, and a plausible, experimentally verified MOA explaining such effects without systemic distribution of formaldehyde is lacking.

- The review of the reproductive and developmental outcomes in the draft IRIS assessment includes relevant outcomes and literature. It does not consistently provide a critical evaluation of the quality of publications and data presented or note strengths and weaknesses of each study. That is especially the case with the animal studies (p. 108).

  Response: In the current assessment, the epidemiological and animal toxicological studies of reproductive and developmental outcomes were evaluated and synthesized using a common framework applied to all hazard categories and outcomes. The studies are described in tables categorized according to confidence in the study results determined by systematic evaluation of study quality, risk of bias and sensitivity. The study evaluations, with the strengths and limitations of the studies, are documented in supplemental material (see Appendix A.5.3). The evidence integration section provides the summary rationale supporting the hazard judgment.

- Animal data also suggest an effect, but EPA should weigh the negative and positive results rigorously inasmuch as negative results outnumbered positive ones for some end points, should evaluate study quality critically because some studies of questionable quality were used to support conclusions, and should consider carefully potential confounders, such as maternal toxicity, effects of stress, exposure concentrations above the odor threshold, and potential for oral exposures through licking (p. 10).

  Response: The text and tables in Appendix A.5.8 describe the criteria used to evaluate the animal studies and the level of information provided by each study to the assessment of hazard, in light of strengths and limitations. Considerations included maternal toxicity, effects of stress, exposure concentrations above the odor threshold and potential for oral exposures through licking. A key consideration for the interpretation of developmental and reproductive outcomes associated with inhalation exposures to formaldehyde was the potential for co-exposure to methanol, a known developmental and reproductive toxicant, when the test article was an aqueous solution of formaldehyde. Studies that used formalin but did not control for methanol, and studies that did not characterize the formaldehyde source, are identified throughout. Such studies were assigned a "low" confidence rating. The consistency of study results with regard to specific outcomes was a key consideration in the synthesis and integration of evidence, which describes and then weighs the available evidence based on the evidence integration considerations (including consistency in results) presented in the Preface.

- The rationale for the assessment of the body of the epidemiologic evidence as convincing is not well articulated. Issues regarding the potential portal of entry and mode of action in relation to reproductive and developmental outcomes are not integrated into the weight-of-evidence discussion (p. 108).

Response: The evaluation of hazard for reproductive and developmental outcomes in the current draft assessment was conducted using an approachfor study evaluation and evidence integration developed for the assessment. The evidence was integrated across the human, animal and mechanistic streams of evidence.

- Although the epidemiologic studies provide only a suggestive pattern of association, EPA followed its guidelines and chose the best available study to calculate a candidate RfC (p. 10). The point of departure is appropriately selected (p. 108).

Response: EPA agrees with this comment.

Lymphohematopoietic cancers

- EPA evaluated the evidence of a causal relationship between formaldehyde exposure and several groupings of LHP cancers—"all LHP cancers," "all leukemias," and "myeloid leukemias." The committee does not support the grouping of "all LHP cancers" because it combines many diverse cancers that are not closely related in etiology and cells of origin. The committee recommends that EPA focus on the most specific diagnoses available in the epidemiologic data, such as acute myeloblastic leukemia, chronic lymphocytic leukemia, and specific lymphomas (pp. 11 and 113).

Response: EPA agrees with this recommendation. The current hazard assessment focuses on the specific diagnoses of myeloid leukemia, lymphatic leukemia, multiple myeloma, and Hodgkin lymphoma, and does not draw conclusions for the broad categories of "all leukemias," grouping of nonspecific lymphomas, or "all LHP cancers."

- As with the respiratory tract cancers, the draft IRIS assessment does not provide a clear framework for causal determinations. As a result, the conclusions appear to be based on a subjective view of the overall data, and the absence of a causal framework for these cancers is particularly problematic given the inconsistencies in the epidemiologic data, the weak animal data, and the lack of mechanistic data. Although EPA provided an exhaustive description of the studies and speculated extensively on possible modes of action, the causal determinations are not supported by the narrative provided in the draft IRIS assessment. Accordingly, the committee recommends that EPA revisit arguments that support determinations of causality for specific LHP cancers and in so doing include detailed descriptions of the criteria that were used to weigh evidence and assess causality (pp. 11 and 113).

Response: Consistent with causal evaluations for all of the health effects, the sets of epidemiologic studies related to each cancer type were evaluated using a common evidence integration framework for determinations of causality that is explicitly described in the Preface. The causal determinations for cancer in the current draft are consistent with EPA's cancer guidelines.

- Clarify how EPA determined weight and strength of evidence. The draft assessment should be revised to discuss the benefits, limitations, and justifications of using one exposure metric to determine causality and another to calculate cancer unit risk. Because the draft assessment relies solely on epidemiologic studies to determine causality, further discussion of the specific strengths, weaknesses, and inconsistencies in several key studies is needed. As stated in EPA's cancer guidelines, EPA's approach to weight of evidence should include "a

single integrative step after assessing all of the individual lines of evidence" (U.S. EPA, 2005, Section 1.3.3, p. 1-11). Although a synthesis and summary are provided, the process that EPA used to weigh different lines of evidence and how that evidence was integrated into a final conclusion are not apparent in the draft assessment and should be made clear in the final version.

Response: As described in the response to related comments on respiratory tract cancers, the sets of studies related to each cancer type were evaluated using a common evidence integration framework for determinations of causality and the rationales are described in the integrated summaries of evidence in Sections 1.3.3 of the Toxicological Review. The determination of causality was based on multiple epidemiologic studies that found associations with different exposure metrics, and which were supported by mechanistic studies in exposed humans that provided biological support for genotoxic and immunologic changes in peripheral blood cells. The epidemiological and human mechanistic evidence was synthesized and strength of evidence judgments were drawn using the framework for human evidence in the Preface. This strength of evidence judgment was integrated with the available animal and other mechanistic evidence, although the results from these studies were largely null. This process is consistent with EPA's cancer guidelines. The rationale for EPA's selection of the exposure metric used to derive a quantitative estimate is provided in Section2.2.2).

- Revisit arguments that support determinations of causality of specific LHP cancers and in so doing include detailed descriptions of the criteria that were used to weigh evidence and assess causality. That will add needed transparency and validity to the conclusions.

Response: The synthesis of the epidemiological evidence for specific LHP cancers uses a common framework for determinations of causality that was developed for the assessment.

- If EPA decides to rely on meta-analysis as a tool to assess causation, it should perform its own meta-analysis with particular attention to specific diagnoses and to variables selected and combined for analysis. The contrasting conclusions of the published meta-analyses make it difficult to rely on conclusions from any one analysis (see, for example, (Bachand et al., 2010; Schwilk et al., 2010; Zhang et al., 2009)) (p. 113).

Response: EPA agrees that the contrasting conclusions in the published meta-analyses make it difficult to rely on conclusions from any one analysis. EPA does not rely on the conclusions of published meta-analyses.

Quantitative assessment

- The committee supports EPA's selection of effects on which it based candidate RfCs but does not support the advancement of two studies selected by EPA: Ritchie and Lehnen (1987) and Rumchev et al. (2002). Furthermore, the lack of clear selection criteria, inadequate discussion of some modes of action, little synthesis of responses in animal and human studies, and lack of clear rationales for many conclusions weaken EPA's arguments as presented in the draft IRIS assessment.

Response: The current draft assessment is based on a defined structure with criteria for systematic review and the integration of evidence to determine causality. The dose-response assessment (see Section 2) also is based on a defined structure with criteria for

selecting studies for the derivation of candidate RfCs and organ-specific RfCs. The studies by Ritchie and Lehnen (1987) and Rumchev et al. (2002) were not used to derive RfCs for reasons described in the hazard assessment.

- The committee disagrees with EPA's decision not to calculate a candidate RfC for upper respiratory tract pathology. Many well-documented studies have reported the occurrence of upper respiratory tract pathology in laboratory animals, including nonhuman primates, after inhalation exposure to formaldehyde, and the committee recommends that EPA use the animal data to calculate a candidate RfC for this end point.

  Response: EPA agrees with this point and has evaluated the toxicology studies reporting respiratory tract pathology to identify a POD and derive a candidate RfC based on incidence of squamous metaplasia (Woutersen et al., 1989; Kerns et al., 1983) (see Section 2.1.2).

- The committee found that EPA dismissed the results of the exposure chamber and other nonresidential studies too readily. Although the exposure durations for the chamber studies are short relative to the chronic duration of the RfC, the studies provide complementary information that could be used for deriving a candidate RfC.

  Response: EPA agrees that the controlled human exposure studies provide complementary information and relied on these studies in concert with the occupational and residential studies to establish formaldehyde as a sensory irritant. The data indicate that this response may be a more immediate phenomenon. In accordance with the criteria for selecting studies for the derivation of candidate RfCs, EPA ultimately selected the dose-response information for sensory irritation from epidemiology studies of residential exposure because these studies evaluated populations including a range of ages, males and females, and with health conditions perhaps conferring susceptibility (Liu et al., 1991; Hanrahan et al., 1984) and compared these to cRfCs derived from medium confidence controlled human exposure studies (Kulle, 1993; Andersen and Molhave, 1983). For other effects, controlled human exposure studies of acute effects after exposures of minutes or hours did not contribute to the evaluation of dose response and development of RfCs. However, evidence from controlled human exposure studies was synthesized in the hazard assessments for pulmonary function, immune-mediated conditions, and nervous system effects.

- Regarding the uncertainty factor that accounts for variability in response of the human population, the committee suggests application of a value of 3 to calculate the candidate RfCs on the basis of the work of Garrett et al. (1999a), Hanrahan et al. (1984), and Liu et al. (1991). Those studies included potentially susceptible populations, so the default value of 10 is not necessary. However, uncertainties remain regarding susceptible populations and factors that affect susceptibility, so a value of 1 is not recommended.

- **Response:** Notably, the format and approach towards deriving candidate RfCs presented in the 2010 draft are substantially different in the current draft. Currently, organ- or system-specific RfCs corresponding to each health outcome with credible evidence of hazard (e.g., sensory irritation; pulmonary function) are being separately derived, in addition to an overall RfC. The derivation of the cRfCs, with the application and rationales for UFs, including different UF_Hs for different cRfCs, is documented in Section 2.1 of the toxicological review.

- Regarding the uncertainty factor that accounts for database completeness, the committee suggests that EPA apply its first option as described in the draft IRIS assessment; that is, apply a value of 1 with the qualification that further research on reproductive, developmental, neurotoxic, and immunotoxic effects would be valuable.

  Response: EPA selected a database uncertainty factor of 1 with the qualification that further research is needed for several health endpoints.

- Although there are some gaps in the data on reproductive, developmental, immunologic, and neurotoxic effects, the likelihood that new effects will be observed at concentrations below those at which respiratory effects have been observed is low. Thus, the committee supports the use of a UFD of 1 with the caveat that research of the types noted should be pursued (p. 9).

  Response: Thank you for the recommendation. EPA selected a database uncertainty factor of 1 with the qualification that further research is needed for several health endpoints.

- Overall, the committee found little synthesis of the relationships among the identified noncancer health effects; it appeared that EPA was driven by the need to identify the best study for each health effect rather than trying to integrate all the information. The committee strongly recommends the use of appropriate graphic aids that better display the range of concentrations evaluated in each published study selected for quantitative assessment; the figures may help to identify how findings of studies cluster and especially identify low or high reference values that may be inconsistent with the body of literature. Ultimately, such graphics will improve the ability of the assessment and make a compelling case for the RfC ultimately put forward.

  Response: The current draft presents the candidate RfCs together, including the relevant PODs and the uncertainty factors applied. In addition, the rationale for selecting the overall RfC from the organ/system-specific RfCs includes a scatterplot of the organ/system-specific RfCs in relation to the average composite UFs applied to derive each one, with the highest uncertainty factors at the bottom of the graph. The size of the symbols for each organ/system RfC represents confidence in the study(ies), POD(s) and database. In this way, the larger RfCs grouped closer to the top of the graph are associated with higher certainty.

- Regarding calculation of unit risks, the committee agrees that the NCI studies and the findings of the two follow-ups are a reasonable choice because they are the only ones with sufficient exposure and dose-response data for risk estimation. However, the studies are not without their weaknesses, and these need to be clearly articulated in the revised IRIS assessment.

  Response: The current draft assessment includes a structured presentation of the limitations and strengths of the epidemiology studies of cancer found in the supplemental material (see Appendix A.5.9) and discussed as appropriate in the synthesis of the evidence in Sections 1.2.5,1.3.3, and 2.2.2, the latter of which outlines these strengths and limitations in the context of uncertainties in the unit risk estimates.

- The committee agrees that EPA's choice of NPC, Hodgkin lymphoma, and leukemia data from the NCI studies to estimate a unit risk is appropriate given that the analysis of Hodgkin

lymphoma and leukemia primarily supports the assessment of uncertainty and the magnitude of potential cancer risk. However, the mode of action for formaldehyde-induced Hodgkin lymphoma and leukemia has not been clearly established. Moreover, the highly limited systemic delivery of formaldehyde draws into question the biologic feasibility of causality between formaldehyde exposure and the two cancers. Thus, substantial uncertainties in using Hodgkin lymphoma and leukemia for consensus cancer risk estimation remain.

Response: The hazard descriptor, *carcinogenic to humans*, is independently substantiated by three evidence integration judgments, namely that the **evidence demonstrates** that formaldehyde inhalation causes nasopharyngeal cancer, sinonasal cancer and, myeloid leukemia, in exposed humans, given appropriate exposure circumstances. These conclusions were based on the currently available evidence using the approaches described in the Preface, which included a specific and explicit consideration of mechanistic evidence when drawing each conclusion. For myeloid leukemia, the assessment acknowledges that, while the human evidence alone supports the strongest causal conclusion, no MOA has been established to explain how formaldehyde inhalation causes this type of cancer without systemic distribution. However, consistent with EPA guidelines and IRIS assessment practice, this lack of MOA understanding does not weaken the human evidence. Section 1.3.3 discusses in depth the uncertainties associated with each causality conclusion.

The uncertainties in use of the available myeloid leukemia data for deriving unit risk estimates are outlined in Section 2.2.2. These uncertainties do not relate to the biologic feasibility of causality for myeloid leukemia. Given the strength of the hazard determination, based on EPA guidelines and IRIS assessment practice, a unit risk estimate for myeloid leukemia would typically be developed and included in the final toxicity value. Ultimately, however, due to complications in the only dataset amenable to dose-response analysis, the current assessment does not include the myeloid leukemia estimate in the IUR. An estimate for myeloid leukemia is developed and presented in the assessment, the uncertainties are transparently outlined, and the development and use of this estimate (e.g., either not at all, in the IUR, or to inform uncertainty) is posed as an explicit charge to the external peer reviewers.

- The draft IRIS assessment does not provide adequate narratives regarding selection of studies and end points for derivation of unit risks. The committee strongly recommends that EPA develop, state, and systematically apply a set of selection criteria for studies and cancer end points. The committee recognizes that uncertainty and variability remain critical issues as EPA continues to promote quantitative assessment to improve environmental regulation. There are still technical gaps in developing and applying quantitative analysis of uncertainty and variability, especially to incorporate from all sources and at all stages into an overall summary. The NRC Committee to Review EPA's Toxicological Assessment of Tetrachloroethylene (NRC, 2010) made several recommendations for advancing methodology and promoting applications. Further research is needed to study various approaches. Small (2008) discussed a probabilistic framework. Given a set of options related to a key assumption (such as mode of action) or a key choice (such as cancer end point), a preference score (or prior probability) may be assigned to each option. The final risk estimate thus also has a weight or probability attached that combines the preference on all options over each assumption or choice. The overarching weight is the result of propagation of uncertainty in each assumption or choice and aggregation of all assumptions over the risk assessment process tree. The collection of

final risk estimates for all permissible combinations of assumption and choice forms an empirical distribution. That distribution quantifies the full range of variation and uncertainty in the risk estimate. With the full range of variation of risk estimates and other information on preference of key assumptions and choices, regulatory policy can depend less on a single principal study, a single principal dataset, or a principal end point. The risk-management process may use the distributional properties of the risk estimate to choose a final risk estimate in the context of all feasible assumptions and choices. The committee concludes that further development of systematic approaches to quantifying uncertainty and variation will enable EPA to conduct IRIS assessments in a more transparent and objective fashion (pp. 107–108).

Response: Thank you for the description of possible approaches to quantifying uncertainty and variation in deriving unit risk estimates. The Agency is working on developing methods to better quantify uncertainty although no validated approaches have been offered to date. The current draft presents a number of sensitivity analyses that examine a range of unit risk estimates associated with different assumptions. As described in prior responses, the current draft presents and applies criteria for systematically considering and selecting endpoints and exposure metrics for quantitative analyses and includes thorough discussions of the inherent uncertainties in the estimates that are presented.

- Derivation of RfC: Overall, the committee is troubled by the presentation and derivation of the proposed RfC values and strongly recommends the approach illustrated and described in Figure S-1. A similar approach was recommended by the NRC Committee to Review EPA's Toxicological Assessment of Tetrachloroethylene and used in recent EPA assessments of tetrachloroethylene and trichloroethylene. Appropriate graphic aids that enable the visualization of the concentration ranges of the candidate RfCs may identify a central value, isolate especially low or high RfC values that might not be consistent with the body of literature, and ultimately improve the ability of the assessment to make a compelling case that the RfC proposed is appropriate for the most sensitive end point and protective with regard to other potential health effects (p. 13).

  Response: The current assessment follows a process complementary to that outlined in Figure S-1 of the NAS review (p. 13). This is the systematic review process developed for the formaldehyde assessment and described in the Preface to the toxicological review. The criteria and rationale for identifying studies with appropriate data for deriving a cRfC are found in Chapter 2 of the assessment and a figure is included that summarizes the cRfCs for each hazard with the range of concentrations that span the POD to the cRfC. The current assessment also derives organ-specific RfCs (providing the rationale for their derivation), and includes a scatterplot of the organ/system-specific RfCs, which both aid in providing the rationale for selection of the overall RfC.

- Regarding calculation of unit [cancer] risks: The committee agrees that the NCI studies are a reasonable choice because they are the only ones with exposure and dose-response data sufficient for calculation of the unit risks; however, the studies are not without their weaknesses, which should be clearly discussed and addressed in the revised IRIS assessment. Although there are uncertainties as discussed above regarding the causal relationship of formaldehyde exposure and the three kinds of cancer, EPA's decision to calculate unit risk values for them appears to be defensible on the basis of the Agency's cancer guidelines. However, EPA should provide a clear description of the criteria that it

used to select the specific cancers and demonstrate a systematic application of the criteria (p. 10).

Response: EPA has clarified its discussion of the NCI studies strengths and limitations (see Section 2.2 of the Toxicological Review). The evaluation of cancer types also is expanded, as is the rationale for selection of cancer types for evaluation of dose-response relationships.

- The calculation of the unit risk values is a complex process, involves many sources of uncertainty and variability, and is influenced by the low-dose extrapolation used (for example, linear vs threshold). The committee therefore recommends that EPA conduct an independent analysis of the dose-response models to confirm the degree to which the models fit the data appropriately. EPA is encouraged to consider the use of alternative extrapolation models for the analysis of the cancer data; this is especially important given the use of a single study, the inconsistencies in the exposure measures, and the uncertainties associated with the selected cancers (p. 10).

- Overall, the committee finds EPA's approach to calculating the unit risks reasonable. However, EPA should validate the Poisson dose-response models for NPC, leukemia, and Hodgkin lymphoma mortality with respect to adequacy of model fit, including goodness of fit in the low-dose range, (log) linearity, and absence of interactions of covariates with formaldehyde exposure. Furthermore, EPA is strongly encouraged to conduct alternative dose-response modeling by using Cox regression or alternative nonlinear function forms.

Response: EPA conducted an independent analysis of the dose-response models to confirm model fit to data.

Analytical results quantifying exposure-response relationships were available from the occupational cohort study conducted by NCI. The published studies provided information about the Poisson dose-response models used to evaluate cancer mortality, including which exposure metrics were evaluated, the p-values for exposure-response trend, and the additional covariates and interaction terms included in the models (Beane Freeman et al., 2013; Beane Freeman et al., 2009; Hauptmann et al., 2004b).

Additional information describing the model covariates and the impact of different model forms (e.g., different lag periods, inclusion of terms for coexposures) on the magnitude or statistical significance of the association of the exposure terms with mortality has been added to the description of the studies in the assessment.

NCI described in the published papers their approach to model evaluation, which included evaluating the models in the entire cohort (nonexposed and exposed) and only among the exposed workers, evaluating multiple possible lag periods, and adding quadratic terms to explore whether such terms indicated significant deviation from a log-linear relationship. EPA concluded that the approach and level of reporting detail in the papers was acceptable and obtained from the NCI the regression coefficients for the trend models reported in the papers. NCI informed EPA that after publication of the 2003 and 2004 papers, independent investigators obtained the cohort data and were able to recreate the results using these models. In addition, for the most recent follow-up of the cohort, with deaths through 2004, the NCI convened a group of extramural scientists to provide advice on the protocol for how to conduct the follow-up. At that meeting, the NCI proposed to use the same methodologies for analysis as in the prior publications. For the 2009 publication, regression models using

the same covariates as the 2003 and 2004 publications were built. In addition, two researchers independently ran all analyses to confirm that no errors had inadvertently been introduced. NCI's extensive internal review processes serve as additional layers of verification and validation above and beyond peer review.

The following detail on the covariates included in the Poisson regression models was added to the assessment. The Poisson regression models stratified the cohort by calendar year (5-year categories), age (5-year categories), sex, and race (white or other) and adjusted for pay category (salary, ever wage, or unknown) (Beane Freeman et al., 2013; Beane Freeman et al., 2009; Hauptmann et al., 2004b). Multiple lag lengths in exposure were assessed and the goodness of fit did not differ substantially for the different lag lengths; a 15-year lag was selected by NCI for solid tumors and a 2-year lag for the lymphohematopoietic cancers. Eleven potential confounding exposures (including benzene) in the plants were evaluated by NCI and found not to alter the RR estimates appreciably in any of the models.[36] Additionally, to specifically rule out an effect of benzene on the lymphohematopoietic cancer results, individuals with possible exposure to benzene were excluded from the analysis, and this did not change the RR estimates. As a final check on the potential for confounding, Hauptmann et al. (2004b) noted that evidence suggests that smoking is not a confounder because there was no consistent excess or deficit for other tobacco-related diseases, for example, bladder cancer, emphysema, and ischemic heart disease. The careful work by NCI to evaluate the potential for confounding is considered sufficient to confirm that the models fit the data appropriately.

The NAS comment and recommendation above refers to the evaluation of model fit, and our response assumes that the NAS panel is concerned specifically with whether the exposure term in the model adequately fits the data. For the log-linear model, the *p*-value for a trend test for the exposure metric in the model indicates the degree to which the log of relative risk rises (or falls) with increases in the exposure metric.

The p-values for the tests for trend for each exposure metric were reported in the published papers. From the 2004 follow-up, the p-values using the cumulative exposure term (ppm-years) indicated that an increasing trend in cancer relative risk was observed for NPC (p = 0.07), leukemia (p = 0.08), and Hodgkin lymphoma (p = 0.06). The p-values for average intensity (ppm) indicated a rising trend in relative risk only for Hodgkin lymphoma (p = 0.03). Finally, the p-values for peak exposure (4 categories [ppm]) indicated a rising trend in relative risk for leukemia (p = 0.02), myeloid leukemia (p = 0.07) and Hodgkin lymphoma (p = 0.004).

- One may also wonder whether there were any covariates (such as sex) that interacted with formaldehyde exposure. The presence of any interactions that indicate effect modification will make the extra risk formula (Rx – Ro/(1 – Ro) depend on the covariates involved rather than independent, as assumed in the draft IRIS assessment" (pp. 137–139).

Response: Whether or not the association of mortality with formaldehyde exposure varies according to certain characteristics such as age, gender, race/ethnicity, or other individual attributes is an important question in assessing risk. Effect modification by the above

[36]The one exception was a model for NPC that included melamine– note that melamine can be combined with formaldehyde to form a resin and controlling for melamine in an analysis of formaldehyde may essentially be controlling for formaldehyde, therein resulting in an alteration of the RR.

factors was evaluated by NCI. According to Beane Freeman et al. (2009), page 755, "We found no evidence of heterogeneity of relative risks by race (white or other), sex, or pay category (salaried or hourly)." The evaluation of effect modification (evaluated statistically using a cross-product term in the model) was conducted for the lymphohematopoietic cancer types under study, including myeloid leukemia and multiple myeloma, and for all exposure metrics. Likewise, Hauptmann et al. (2004b) tested heterogeneity for the solid cancers and did not report any significant heterogeneity (see Table 7). Therefore, it was not necessary to account for variation in risk by these individual characteristics in the estimation of the unit risk.

- EPA is encouraged to consider the use of alternative extrapolation models, including Cox regression models and nonlinear model forms. The details of such modeling activities should be included in an appendix to the IRIS assessment in sufficient detail that the results can be reproduced…The authors (Callas et al., 1998) suggested that Cox regression be used when confounding cannot be well controlled or when age at cancer death does not follow an exponential distribution (p. 138).

  Response: EPA agrees that the Cox proportional hazards model is an alternative to the Poisson model; however, because age was carefully controlled in the analyses, the Poisson regression results would be essentially the same as those that would be obtained from a Cox analysis. Callas et al. (1998, 1996) have reported, based on analyses of an earlier follow-up of the NCI formaldehyde cohort, that these two models yield nearly identical RR estimates and CIs except in situations in which age cannot be closely controlled in the Poisson analysis. The NCI analyses had a very fine level of control for age by using 5-year age groups, a nonparametric approach that controls for potential confounding by age even when the risk function for age may be strongly nonlinear.

  The log-linear Poisson model assumed a linear relationship between log RR and formaldehyde exposure. One of the published papers described NCI's approach to evaluating whether the relation of exposure with mortality was log-linear, or whether nonlinear terms would provide a better fit. This was done by including a quadratic term in the Poisson analysis to investigate whether there was a departure from the log-linear model. The authors concluded that there was no evidence of a departure from log-linearity for NPC (personal communication from Michael Hauptmann, June 11, 2013) and all leukemia (Beane Freeman et al., 2009).

APPENDIX E. SUMMARY OF PUBLIC COMMENTS AND EPA'S DISPOSITION [PLACEHOLDER]

EPA responses to public comments received during the 60-day public commnt period will be added prior to finalizing the assessment.

APPENDIX F. SYSTEMATIC EVIDENCE MAP UPDATING THE LITERATURE FROM 2016–2021

F.1. INTRODUCTION

This systematic evidence map (SEM) updates the literature that was assessed to develop the 2017 Step 1 draft IRIS formaldehyde-inhalation assessment. The completed draft 2017 IRIS assessment was suspended by EPA (https://www.epa.gov/sites/default/files/2019-04/documents/iris_program_outlook_apr2019.pdf) and shared with EPA's OCSPP-OPPT program for use in developing a risk evaluation under TSCA. However, in 2021, development of the IRIS assessment was unsuspended (https://www.epa.gov/sites/default/files/2021-03/documents/iris_program_outlook_mar2021.pdf). This SEM was developed to identify the relevant literature published since the suspension of the 2017 draft, in particular studies that may alter hazard or toxicity value conclusions presented in the 2017 draft. Studies identified in this SEM as possibly impactful to the 2017 draft conclusions have been incorporated into the updated 2021 draft IRIS Toxicological Review.

F.2. METHODS

This SEM identifies and documents the literature relevant to assessing the potential human health hazards of formaldehyde inhalation from January 2016–May 2021. The search terms and screening strategies are nearly identical (exceptions noted later in this document) to those used to develop the 2017 Step 1 draft, and the detailed methods can be found in the Supplemental Information to the Toxicological Review of Formaldehyde – Inhalation (see Appendix A.5). In Appendix A.5, supporting materials for each health effect include tables listing the search terms for each bibliographic database searched, and tables listing the inclusion and exclusion criteria used to search and screen the identified citations (PECO).

F.2.1. Specific Aims

The following specific aims were identified for the SEM.

- Identify epidemiological (i.e., human), toxicological (i.e., experimental animal), and mechanistic literature using an identical literature search approach as was used to develop the 2017 Step 1 draft IRIS formaldehyde-inhalation assessment reporting effects of exposure to formaldehyde as outlined in the health effect-specific PECOs found in Appendix A.5 of the Supplemental Information to the Toxicological Review of Formaldehyde – Inhalation.

- Tag secondary (not primary research) studies.

- Create a literature inventory of PECO-relevant studies. The literature inventory summarizes basic features of study design, health system(s), and endpoints assessed.

- Assess PECO-relevant studies, within each health effect category, to determine if they are possibly impactful to the 2017 draft assessment decisions on hazard and dose response and document the reasons in a literature inventory.

F.2.2. Populations, Exposures, Comparators, and Outcomes (PECO) Criteria and Supplemental Material Tagging

A PECO is used to focus the research question(s), search terms, and inclusion/exclusion criteria used in a SEM or systematic review. For this SEM, health effect-specific PECOs were used for the literature search and screening process and can be found in Appendix A.5 of the Supplemental Information to the Toxicological Review of Formaldehyde – Inhalation. For each health effect, the PECOs list the different populations and endpoints of interest. In addition, PECOs tailored to mechanistic studies were used—these also are found in Appendix A.5 of the Supplemental Information to the Toxicological Review of Formaldehyde – Inhalation. The PECO for lymphohematopoietic (LHP) cancer in animal studies is provided below as an example (Table 1).

In addition to identifying studies that met the PECO criteria and studies that were excluded, tags were added to nonprimary research studies (i.e., reviews, commentaries, letters, etc).

Table F-1. Example of outcome-specific PECO: LHP cancer in animals

PECO element	Description
Populations	**Animal:** Nonhuman mammalian animal species (whole organism) of any lifestage (including preconception, in utero, lactation, peripubertal, and adult stages). In-vitro assays and non-experimental animal studies are excluded.
Exposures	Relevant forms: Formaldehyde (generated from formalin, paraformaldehyde, or other sources) • • **Animal:** Any exposure to formaldehyde via inhalation route[s] of >1 d duration, or any duration assessing exposure during reproduction or development. • • Non-inhalation dosing regimens are excluded for systemic effects (in this SEM).
Comparators	**Animal:** A concurrent control group exposed to vehicle-only treatment and/or untreated control (control could be a baseline measurement).
Outcomes	LHP cancers.

F.2.3. Literature Search and Screening Strategies

Database Searches

To identify relevant studies published since the 2017 draft was developed, separate searches were conducted for the health effect categories listed in Table 2 encompassing January 2016 to May 2021 (overlapping with the search dates of the 2017 draft). Separate searches across two databases were conducted for different health outcomes (e.g., sensory irritation, cancer). In addition to the health effects listed in Table 2, specific search strategies were used to identify literature on additional topics (e.g., toxicokinetics and mechanistic information related to respiratory tract cancers and LHP cancers). While the searches for cancer mechanisms primarily focused on genotoxicity endpoints, the searches for mechanistic research on inflammation and immune effects and respiratory pathology retrieved studies also relevant to cancer. While earlier literature updates included a search strategy on exposure to formaldehyde, this research category was not updated for this search as exposure is not a review topic for the assessment.

The search strategies are identical to those used to develop the 2017 Step 1 draft, which used PubMed, Web of Science and ToxNet, although this update did not include ToxNet, which has not been available since December 2019. Details on the database searches can be found in the Appendix A.5 of the Supplemental Information to the Toxicological Review of Formaldehyde – Inhalation.

Table F-2. Literature search strategy

Databases[a]	Health hazard searches[b]
Web of Science PubMed	(formaldehyde, formalin, paraformaldehyde, OR CASN 50-00-0) AND: Sensory Irritation[c] • Pulmonary Function[c] • Immune-Mediated Conditions, focusing on Allergies and Asthma • Respiratory Tract Pathology in Humans • Respiratory Tract Pathology in Animals • Site-specific cancer in Humans • Upper Respiratory Tract Cancer in Animals • Lymphohematopoietic Cancer in Animals • Mechanistic Studies of Upper Respiratory Tract Cancer, focusing on genotoxicity • Mechanistic Studies of Lymphohematopoietic Cancer, focusing on genotoxicity • Inflammation and Immune Effects (mechanistic information)[d] • Developmental and Reproductive Toxicity • Nervous System Effects

[a]PubMed: http://www.ncbi.nlm.nih.gov/pubmed/, Web of Science: http://apps.webofknowledge.com/WOS_GeneralSearch_input.do?product=WOS&search_mode=.

[b]Specific parameters and keywords for each hazard-specific database search strategy are included in Appendix A.5 of the Supplemental Information to the Toxicological Review of Formaldehyde – Inhalation.

[c]A systematic search strategy was not applied to the database of animal studies on this health outcome. Sensory irritation in animals is a well-described phenomenon. For pulmonary function, there was an extensive set of research studies on humans, and therefore, the few studies on this endpoint in animals were not reviewed.

[d]This separate, systematic literature search was performed to augment the analyses of mechanisms relevant to other health effect-specific searches.

Screening Process

Studies identified from the database searches were imported into DistillerSR software (https://www.evidencepartners.com/products/distillersr-systematic-review-software/) for screening. Both title/abstract (TIAB) and full-text screening were conducted by two independent reviewers and any screening conflicts were resolved by discussion between the primary screeners with consultation by a third reviewer if needed. Conflicts between screeners in applying the supplemental tags were resolved similarly, erring on the side of over-tagging. For citations with no abstract, articles were initially screened based on all or some of the following: title relevance (title should indicate clear relevance), and page numbers (articles two pages in length or less are assumed to be conference reports, editorials, or letters). Eligibility status of non-English studies was assessed using the same approach with online translation tools or engagement with a native speaker used to facilitate screening. Full-text records were sought through the EPA's HERO database for studies screened as meeting PECO criteria or "unclear" based on the TIAB screening. In addition, references that had potential relevance to other health-outcome specific projects were identified and then screened within those projects. Access to the example screening form DistillerSR is available upon request for users who have DistillerSR access.

Although some uncertainties remain, the organization and analyses in the assessment assume that inhaled formaldehyde is not distributed to an appreciable extent beyond the upper respiratory tract to distal tissues; thus, it is assumed that inhaled formaldehyde is not directly interacting with tissues distal to the portal of entry (POE) to elicit systemic effects. Therefore, as a deviation from the literature screening approach applied to develop the 2017 draft, studies of exposure routes not involving inhalation, including in vitro studies involving cells from distal tissues, were not considered to be PECO relevant for this literature update and were excluded. Similarly, it is assumed that formaldehyde does not cause appreciable changes in normal metabolic processes associated with formaldehyde in distal tissues. Thus, studies examining potential associations between levels of formaldehyde (i.e., endogenous formaldehyde) or formaldehyde metabolites in tissues distal to the POE (e.g., formate in blood or urine, brain formaldehyde levels) were excluded for most health outcomes, particularly effects on systemic tissues such as the nervous system and reproductive and developmental effects. However, studies of endogenous formaldehyde and mechanisms with its potential relevance to circulating hematopoietic precursor cells and lymphohematopoietic cancers were considered.

F.2.4. Literature Inventory

Human, animal, and mechanistic studies that met PECO criteria after full-text review were briefly summarized in DistillerSR using a structured data extraction form. Studies were extracted by one team member and the extracted data were quality checked by at least one other team

member. The extraction fields in the forms are available in MS Excel format upon request. See (https://www.epa.gov/iris/forms/contact-us-about-iris) for requestors who have DistillerSR access. The literature inventories were exported from DistillerSR in MS Excel format.

For animal studies, the following information was captured: formaldehyde source, study type (e.g., acute, chronic, developmental), duration of treatment, route, species, strain, sex, exposure levels tested, exposure units, and endpoints assessed.

For epidemiological studies, the following information was summarized: population type (e.g., residential/school based, occupational, other), study design (e.g., cross-sectional, cohort, case-control, ecological, case-report, controlled trial, meta-analysis), study location, lifestage (adults, children/infants), exposure measurement (air sampling, occupational history, other), and endpoints assessed.

For mechanistic studies, the information gathered was dependent on the study type: human in vivo, animal in vivo, in vitro/ex vivo, or dosimetry/pharmacokinetic modeling. For dosimetry/pharmacokinetic modeling references, a summary from the paper's abstract was excerpted. For all types of mechanistic studies, study details and exposure metrics were summarized along with the endpoints assessed.

The inventory also includes a decision and explanation as to whether each relevant study is considered "possibly impactful" (i.e., to the 2017 draft assessment conclusions) or "not impactful," as described below.

Considerations for identifying "possibly impactful" studies

Studies that met the PECO criteria after full text screening were further examined to determine if they could potentially be impactful to the assessment with respect to changing hazard conclusions or toxicity values presented in the 2017 draft. This process relied on information gathered from the literature inventory and expert judgment by two reviewers. General considerations for designating studies as *possibly impactful* are included below, with the specific rationales documented in the SEM study summary tables:

- Studies with chronic or subchronic exposure durations or including exposure during reproduction or development, are generally more impactful than studies with acute or shorter-term exposure durations (e.g., <4 weeks in rodent studies).

- Animal studies with multiple dose groups covering a broad range of dose levels, and specifically including lower exposure levels, are generally more impactful than single-dose studies.

- Animal studies employing exposure to formaldehyde without methanol co-exposure (e.g., generated from paraformaldehyde) and with adequate inhalation exposure administration methods were considered more impactful. Methanol, present in aqueous formaldehyde solutions to inhibit polymerization, is a potential confounder of associations between observed health outcomes and formaldehyde exposure via formalin. The test article used to

generate the formaldehyde atmosphere and controls in experimental studies was an important consideration, particularly for non-respiratory health effects.

- More apical endpoints and those most directly related to the mechanistic uncertainties identified in the 2017 draft as most relevant to drawing hazard or dose-response judgments were considered more impactful. The specifics of this consideration vary depending on the health outcome(s) of interest. In some cases, this relevance determination relates to the potential human relevance of the endpoints, while in others this relates to an ability to infer adversity.

- For human studies, prioritization considerations depended on the health effect category, formaldehyde exposure levels, and the extent of the evidence base supporting the hazard conclusions in the 2017 draft. Studies of noncancer respiratory outcomes identified in the PECOs among residential populations or school-aged children were prioritized over occupational studies, which typically involve higher formaldehyde concentrations. Any study of reproductive or developmental outcomes that conducted an exposure assessment (qualitative or quantitative) for formaldehyde was considered possibly impactful. In addition, with some exceptions documented in the inventory tables, studies of ALS, genotoxicity endpoints, or PECO identified cancer outcomes that conducted an exposure assessment (qualitative or quantitative) for formaldehyde were generally considered possibly impactful.

F.3. RESULTS

F.3.1. Sensory Irritation Effects in Human Studies

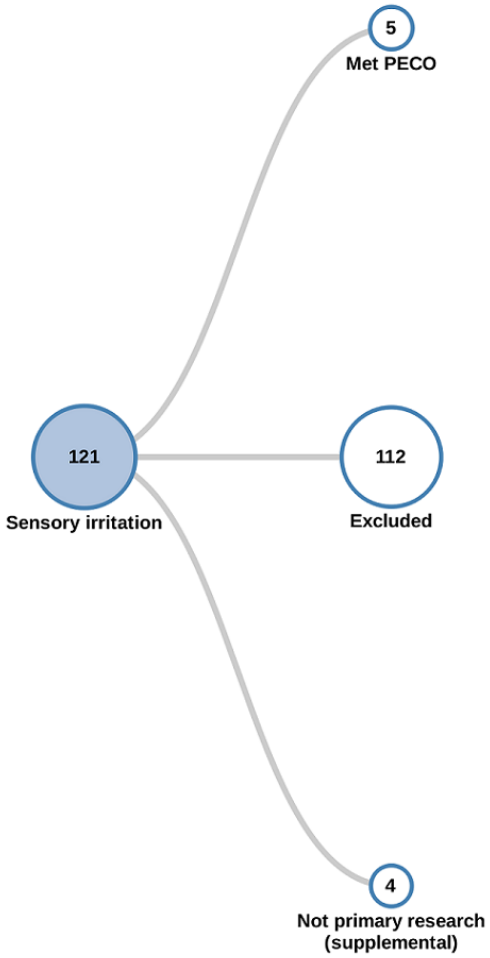


Figure F-1. Sensory irritation literature tree (interactive version here).

A total of 121 citations were retrieved for the assessment of sensory irritation in humans and five studies were PECO-relevant (Table F-3). None of these were deemed to be possibly impactful. Saowakon et al. (2015) already had been included in the 2017 draft.

Table F-3. Studies of sensory irritation effects in humans

Reference	Study design	Exposure	Endpoints	Impact	Rationale
Humans					
Aung et al. (2021)	Occupational Myanmar cross-sectional	Air sampling, adults, medical students and instructors in anatomy dissection rooms	Unpleasant odor, eye irritation, nasal irritation symptoms	Not impactful	High exposure levels, adults, health effects well supported in assessment
Deng et al. (2020) only abstract available (full text Chinese)	Occupational China cross-sectional	Air sampling, adults, medical students in anatomy dissection rooms	Subjective symptoms (e.g., itchy eyes, nasal congestion, runny nose)	Not impactful	High exposure levels, adults, health effects well supported in assessment
Sakellaris et al. (2020)	Occupational Europe (Portugal, Spain, Italy, Greece, France, Hungary, the Netherlands, Finland) cross-sectional	Air sampling, adults, office building occupants	Eye irritation (dry eyes, watering or itchy eyes, burning or irritated eyes), respiratory symptoms (blocked or stuffy nose, runny nose, dry/irritated throat, cough	Not impactful	Adults, health effects well supported in assessment
Saowakon et al. (2015)	Not extracted			Not impactful	Already identified in 2017 draft
Thetkathuek et al. (2016)	Occupational, Chacheongsao Province, Thailand cross-sectional	Air sampling, adults, medium-density fiberboard furniture workers	Respiratory irritation symptoms	Not impactful	High exposure levels, adults, health effects well supported in assessment

Rows for studies judged as "not impactful" are shaded grey.

F.3.2. Pulmonary Function Effects in Human Studies

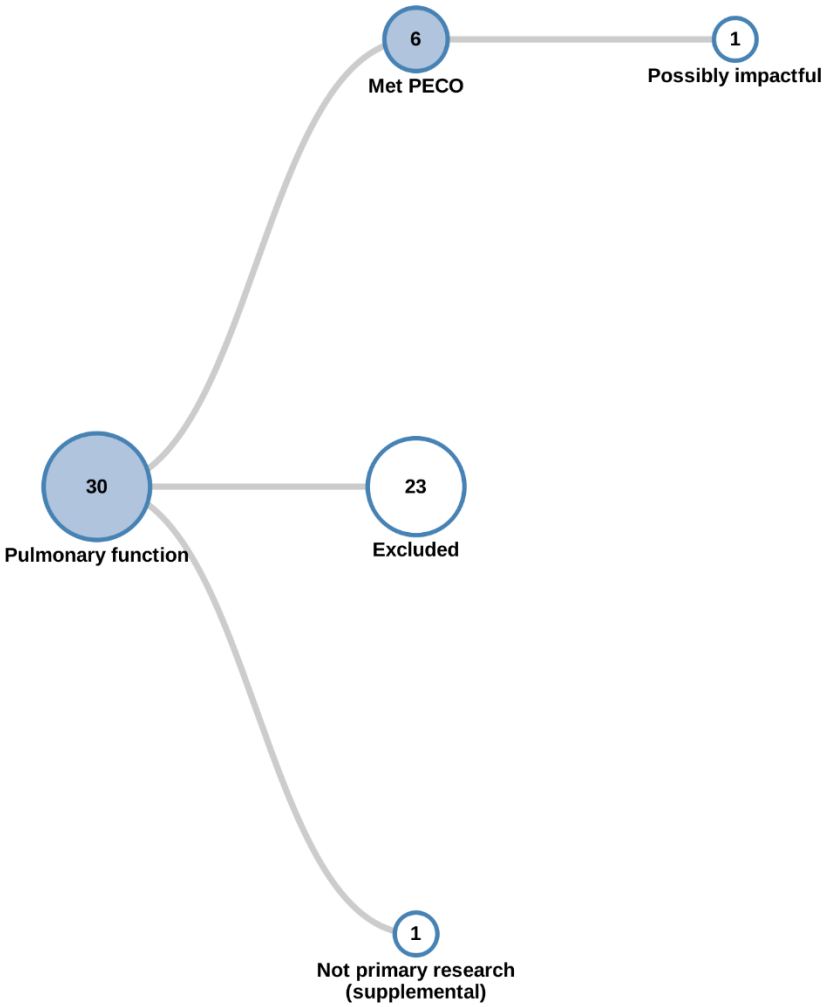


Figure F-2. Pulmonary function effects in humans literature tree (interactive version here).

A total of 30 citations were retrieved for the assessment of pulmonary function effects in humans and six studies were PECO-relevant (Table F-4). Of these, one study, Saowakon et al. (2015), was deemed to be possibly impactful but already had been included in the 2017 draft.

Table F-4. Studies of pulmonary function effects in humans

Reference	Study design	Exposure	Endpoints	Impact	Rationale
Human					
Saowakon et al. (2015)	Not extracted			Possibly impactful	Already identified in 2017 draft
Fsadni et al. (2018)	Schools-based Malta cross-sectional	Air sampling, children, school children	Pulmonary function tests (not specified)	Not impactful	Important details were not provided
Asgedom et al. (2019)	Occupational Ethiopia cross-sectional	Air sampling, adults, particleboard workers	Lung function (FVC, FEV1, FEF 25-75%)	Not impactful	High exposure levels, adults, health effects well supported in assessment
Deng et al. (2020) only abstract available (full text Chinese)	Occupational China cross-sectional	Air sampling, adults, medical students in anatomy dissection rooms	FEV1, FEV1/FVC, PEF, FEF 25%-75%, MEF25%, FEF50%-75%	Not impactful	High exposure levels, adults, health effects well supported in assessment
Neghab et al. (2017)	Occupational Shiraz, Iran cross-sectional	Air sampling, adults, kitchen workers exposed to cooking fumes	VC, FVC, FEV1, PEF, FEV1/FVC, FEV1/VC	Not impactful	High exposure levels, adults, health effects well supported in assessment
Zarei et al. (2017)	Occupational Tehran, Iran cross-sectional	Air sampling, adults, foundry coremakers	FVC, FEV1, FEV1/FVC, peak expiratory flow (PEF), mid forced expiratory flow (FEF25-75%)	Not impactful	High exposure levels, adults, health effects well supported in assessment

Rows for studies judged as "not impactful" are shaded grey; unshaded rows highlight studies incorporated into the updated draft assessment.

$FEF_{25-75\%}$ - mid forced expiratory flow, $FEF_{50-75\%}$ - forced expiratory flow $_{50-75\%}$, FEV_1- Forced expiratory volume in one second, FVC – forced vital capacity, PEF - peak expiratory flow, $MEF_{25\%}$ - mean flow at 25%, VC -vital capacity.

F.3.3. Immune-Mediated Conditions in Humans, Focusing on Allergies and Asthma

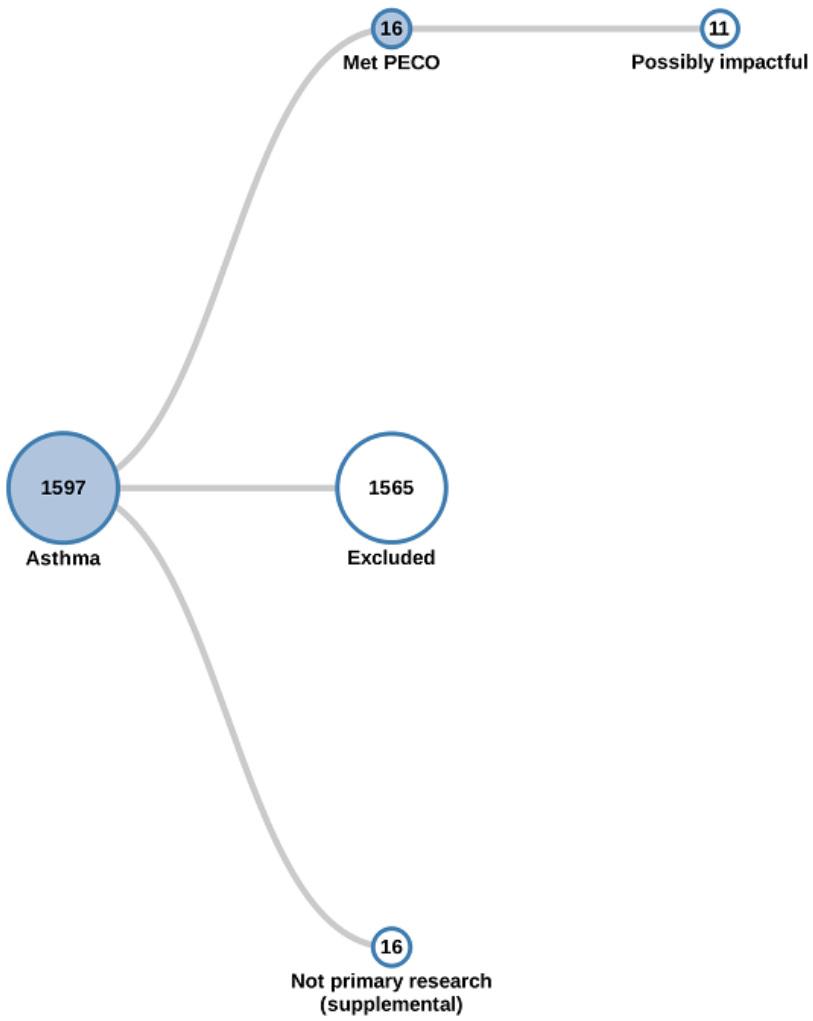


Figure F-3. Asthma and immune effects in humans literature tree (interactive version here).

A total of 1,597 citations were retrieved for the assessment of asthma and immune effects in humans and 16 studies were PECO-relevant (Table F-5). Of these, 11 studies were deemed to be possibly impactful.

Table F-5. Studies of immune-mediated conditions in humans, focusing on allergies and asthma

Reference	Study design	Exposure	Endpoints	Impact	Rationale
Human					
Branco et al. (2020)	School-based Northern Portugal cross-sectional	Air sampling, children, preschoolers/primary school students	Asthma (reported, diagnosed), wheezing (active)	Possibly impactful	School-based – children; indoor formaldehyde concentrations between 10–80 µg/m^3
Huang et al. (2017)	Population-based Shanghai, China case-control	Air sampling in residence, children	Current rhinitis	Possibly impactful	Population-based – children; indoor formaldehyde concentrations between 10–80 µg/m^3
Isa et al. (2020a)	School-based Selangor, Malaysia cross-sectional	Air sampling in classroom, children	Rhinitis (past 12 months), skin allergy (past 12 months)	Possibly impactful	School-based – children; mean indoor formaldehyde concentrations between 10–80 µg/m^3
Lajoie et al. (2014)	Population--based Quebec, Canada intervention study	Air sampling, children, ventilation intervention study	Change in prevalence of asthma symptoms and medical care	Possibly impactful	Population-based – children; mean indoor formaldehyde concentrations between 10–80 µg/m^3
Li et al. (2019)	Population-based Hong Kong cohort	Air sampling, birth to 18 mo	Wheeze (new onset)	Possibly impactful	Population-based – children; mean indoor formaldehyde concentrations between 10–80 µg/m^3
Liu et al. (2018a)	Population--based Changchun, China case-control	Air sampling in residence, children	Asthma diagnosis	Possibly impactful	Population-based – children; indoor formaldehyde concentrations between 10–80 µg/m^3
Madureira et al. (2016)	Population-based Porto, Portugal case-control	Air sampling in residence, children	Current asthma	Possibly impactful	Population-based – children; indoor formaldehyde concentrations between 10–80 µg/m^3
Neamtiu et al. (2019)	School-based Alba County, Romania cross-sectional	Air sampling in classroom, children	Asthma-like symptoms (difficult breathing, dry cough, wheezing in past week), allergy-like symptoms (skin conditions such as rash, itch, eczema; eye disorders such as red, dry, swollen, itching, burning, or sensation of "sand in eyes"; rhinitis such as itching nose, sneezes, stuffy or blocked nose)	Possibly impactful	School-based – children; mean indoor formaldehyde concentrations between 10–80 µg/m^3

Reference	Study design	Exposure	Endpoints	Impact	Rationale
Norbäck et al. (2017)	School-based Johor Bahru, Malaysia cross-sectional	Air Sampling, children	Rhinitis	Possibly impactful	School-based – children; indoor formaldehyde concentrations between 10–80 µg/m^3
Yon et al. (2019)	School-based Seongnam City, Korea cohort	Air sampling in classroom, children	Current asthma, rhinitis, rhinitis severity	Possibly impactful	School-based – children; mean indoor formaldehyde concentrations between 10–80 µg/m^3
Yu et al. (2017)	Population--based Hong Kong cohort	Air sampling in residence, birth to 18 mo	Wheeze (new onset)	Possibly impactful	Population-based – children; mean indoor formaldehyde concentrations between 10–80 µg/m^3
Asgedom et al. (2019)	Occupational Ethiopia cross-sectional	Air sampling, adults, particleboard workers	Respiratory symptoms (cough, cough with sputum production, phlegm, wheezing, shortness of breath)	Not impactful	Occupational exposure - adults, health effects well supported in assessment
Dumas et al. (2020)	Occupational United States cohort	Occupational history and job-task-exposure-matrix, adults, health workers (female nurses)	Self-reported incident physician-diagnosed asthma	Not impactful	Occupational exposure – adults, health effects well supported in assessment
El-Feky et al. (2020)	Occupational Egypt cross-sectional	Industry/ production type, adults, factory workers	Chronic bronchitis, respiratory symptoms and signs, respiratory rate, nasal symptoms, eye symptoms, skin manifestations	Not impactful	Occupational exposure – adults, health effects well supported in assessment
Fsadni et al. (2018)	School-based Malta cross-sectional	Air sampling in classroom, children	Wheezing, rhinitis, eczema, acoustic rhinometry, nasal lavage	Not impactful	Only qualitative results reported, e.g., whether statistically significant and directional arrow
Thetkathuek et al. (2016)	Occupational Chacheongsao Province, Thailand cross-sectional	Air sampling, adults, medium density fiberboard workers	Difficulty breathing, chest discomfort, wheeze	Not impactful	Occupational exposure - adults, health effects well supported in assessment

Rows for studies judged as "not impactful" are shaded grey; unshaded rows highlight studies incorporated into the updated draft assessment.

F.3.4. Respiratory Tract Pathology in Human Studies

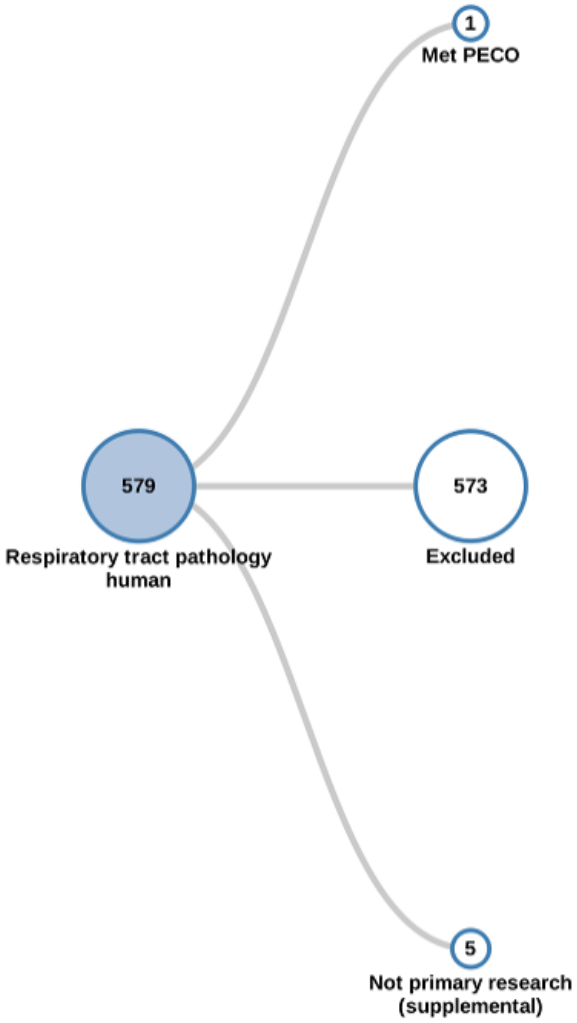


Figure F-4. Human respiratory tract pathology literature tree (interactive version here).

A total of 579 citations were retrieved for the assessment of respiratory tract pathology in humans and one study was PECO-relevant (TableF-6). This study was not deemed to be possibly impactful.

Table F-6. Studies of respiratory tract pathology in humans

Reference	Study design	Exposure	Endpoints	Impact	Rationale
Human					
Bruno et al. (2018)	Occupational Rome, Italy cross-sectional	Air sampling, adults, Laboratory pathology workers	Nasal cytology (muciparous metaplasia)	Not impactful	Adults, health effects well supported in assessment

Rows for studies judged as "not impactful" are shaded grey.

F.3.5. Animal Studies of Respiratory Tract Pathology

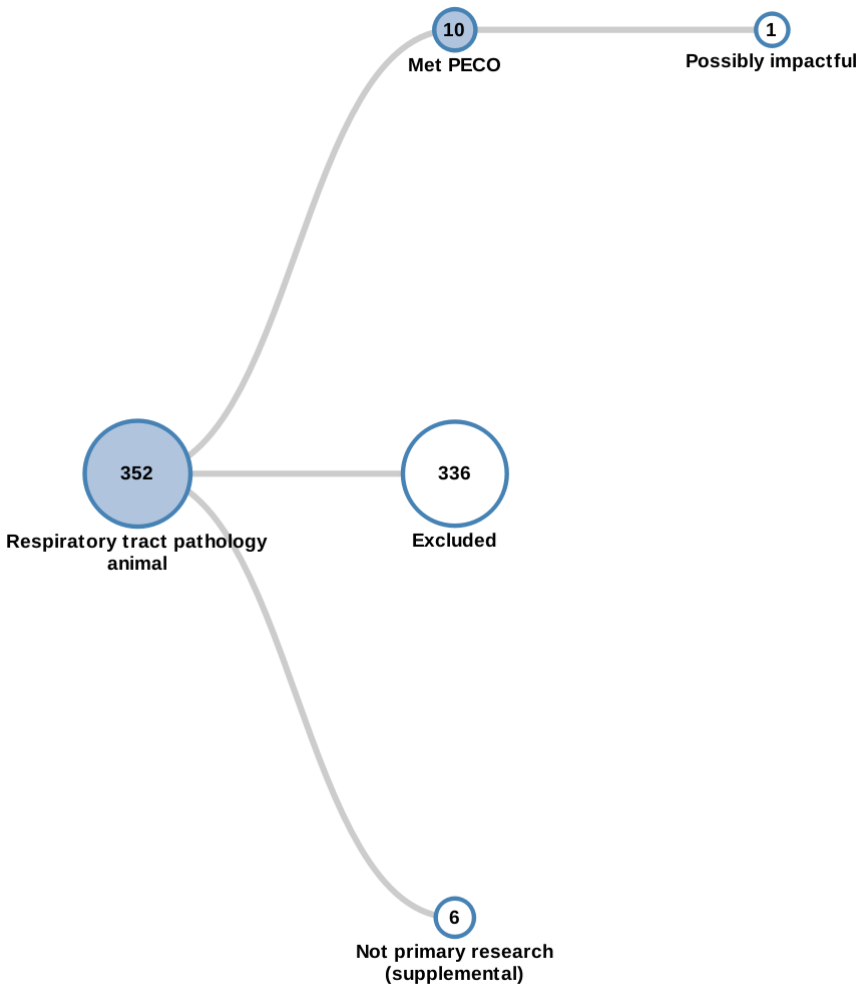


Figure F-5. Animal respiratory tract pathology literature tree (interactive version here).

A total of 352 citations were retrieved for the assessment of respiratory tract pathology in animals and ten studies were PECO-relevant (Table F-7). Of these, one (Morgan et al., 2017) was deemed to be possibly impactful. Although Morgan et al. (2017) was identified in the literature search update and included in the inventory, it already had been included in the 2017 draft Toxicological Review of Formaldehyde-Inhalation.

Table F-7. Animal studies of respiratory tract pathology

Reference	Study design	Exposurea	Endpoints	Impact	Rationale
Animal Studies					
Morgan et al. (2017)	Mouse (Trp53 haploinsufficient), Male Subchronic (8 wk; 6 hr/d, 5 d/wk), then held for 32 wk	Paraformaldehyde 0, 7.5 or 15 ppm (0, 9.2, 18.5 mg/m^3) Inhalation	All major tissues and gross lesions were collected for histopathology (including squamous metaplasia in respiratory tissues)	Possibly impactful	Already included in 2017 draft
Aydemir et al. (2017)	Rat (Wistar), both sexes Subchronic (6 wk; 8 hr/d, 5 d/wk)	Formalin 0, 6 ppm (0, 7.38 mg/m^3) Inhalation	Lung hematoxylin and eosin staining for qualitative review of inflammation and tissue morphology	Not impactful	Formalin
Cheng et al. (2016)	Mouse (Kunming), male Short-term (up to 7 d; continuous)	Formalin 0, 0.08, 0.8 mg/m^3 Inhalation	Hematoxylin and eosin staining for inflammation and edema	Not impactful	Formalin; not key endpoints
Abreu et al. (2016)	Mouse (C57BL/6), both sexes Acute (8 hr)	Unspecified test article 0, 0.2, 1.0, 3.0 ppm (0, 0.25, 1.23, 3.69 mg/m^3) Inhalation	Lung morphology and nasal ciliation; histological inflammatory cell counts in lung and scoring in nose	Not impactful	Unknown test article; acute
Lima et al. (2015)	Rat (Fischer), male Short-term (5 d; 20-min × 3/d)	Unspecified test article 0, 1, 5, 10% Inhalation	Trachea histology and morphometric analyses, including mucus production	Not impactful	Unknown test article; high levels; brief exposures
Liu et al. (2018b)	Rat (Sprague Dawley), male Short-term (4 wk; 8 hr/d)	Formalin 0, 0.5, 5, 10 mg/m^3 Inhalation	Lung histopathological architecture measurements	Not impactful	Formalin; not key endpoints
Payani et al. (2019)	Rat (Wistar), male Short-term (21 d; 1 hr/d)	Unspecified test article 0, 40% Inhalation (vapor)	Pulmonary histopathology	Not impactful	Unknown test article; high levels; brief exposures

Reference	Study design	Exposurea	Endpoints	Impact	Rationale
Sapmaz et al. (2017)	Rat (Sprague Dawley), male Short-term (4 wk; 8 hr/d) or Subchronic (13 wk; 8 hr/d)	Paraformaldehyde 0, 5, 10 ppm (0, 6.2, 12.3 mg/m^3) Inhalation	Hematoxylin and eosin staining (airway inflammation; morphology; scored injury); trachea thickness	Not impactful	Not key endpoints
Sholapuri et al. (2020)	Rat (Wistar), male Short-term (21 d; 1 hr/d)	Formalin 0, 40% Inhalation	Lung histopathology	Not impactful	Formalin; high levels; brief exposures
Song et al. (2017)	Mouse (Balb/c), male Short-term (18 d; 3hr/d)	Formalin 0, 2.44 ppm (0, 3.00 mg/m^3) Inhalation	Airway inflammation histology	Not impactful	Formalin; No formaldehyde-only control (without ovalbumin [OVA])

Rows for studies judged as "not impactful" are shaded grey; unshaded rows highlight studies incorporated into the updated draft assessment.

[a] Use of methanol-stabilized formalin was inferred in some studies based on study-specific description (e.g., 37% stock solution).

F.3.6. Site-specific Cancer in Human Studies

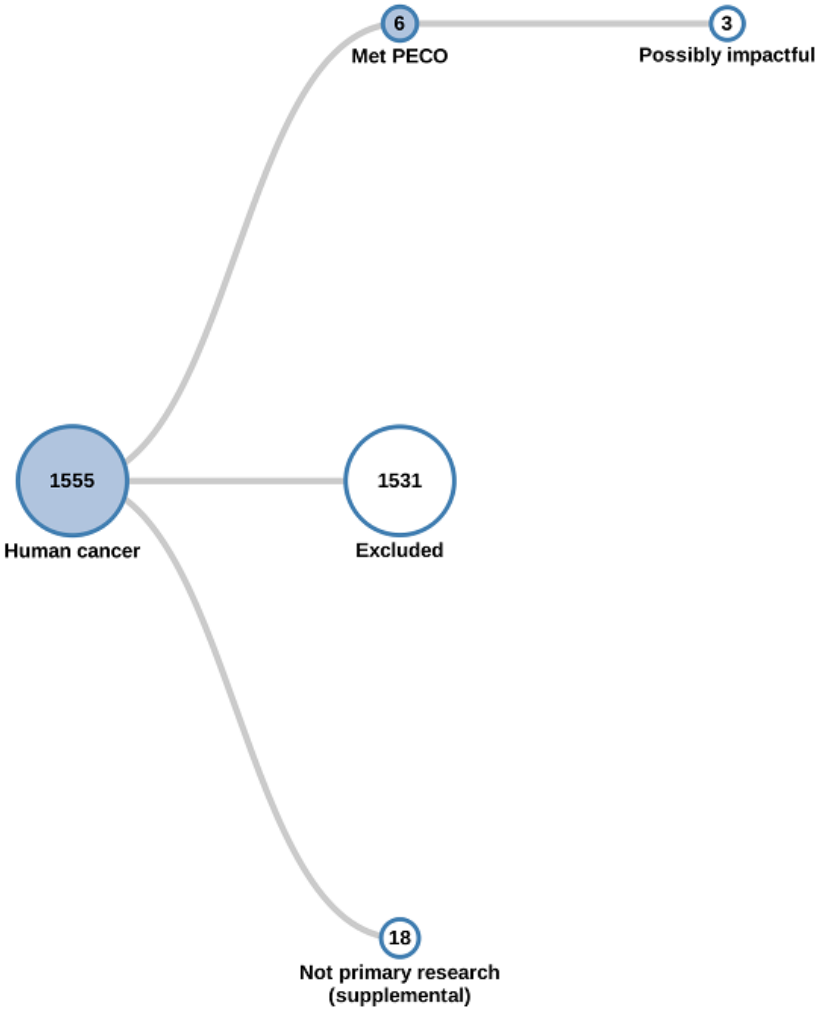


Figure F-6. Human cancer literature tree (interactive version here).

A total of 1,555 citations were retrieved for the assessment of cancer in humans and 6 studies were PECO-relevant (Table F-8). Of these, half (three studies) were deemed to be possibly impactful. Checkoway et al. (2015) and Pira et al. (2014) had been included in the 2017 draft.

Table F-8. Studies of site-specific cancer in humans

Reference	Study Design	Exposure	Endpoints	Impact	Rationale
Human					
Checkoway et al. (2015)	Occupational United States cohort	Air sampling, occupational history, and job-exposure matrix, adults, NCI cohort reanalysis	Cause-specific mortality [non-Hodgkin lymphoma mortality, chronic lymphocytic leukemia mortality, Hodgkin lymphoma mortality, multiple myeloma mortality, myeloid leukemia mortality, acute myeloid leukemia mortality, chronic myeloid leukemia mortality, all leukemia mortality, lymphohematopoietic cancer mortality]	Possibly impactful	Already identified in 2017 draft
Marsh et al. (2016)	Occupational United States cohort	Air sampling, occupational history, and job-exposure matrix, adults, NCI cohort NPC reanalysis	Nasopharyngeal cancer mortality	Possibly impactful	Additional analyses of important studies in the 2017 draft
Möhner et al. (2019)	Occupational United States cohort	Occupation-based, adults, NCI cohort analysis	Mortality from nasopharyngeal cancer [oropharynx, nasopharynx, hypopharynx, pharynx, pharynx (unspecified)]	Possibly impactful	Additional analyses of important studies in the 2017 draft
Pira et al. (2014)	Occupational Piedmont, Italy cohort	Occupational history, adults, laminated plastics workers	Cause-specific mortality [lymphoma, myeloma, leukemia, all lymphatic and hematopoietic tissue neoplasms]	Not impactful	Already identified in 2017 draft
Sernia et al. (2016)	Occupational Italy cohort	Current occupation, adults, university laboratory workers	NPC, leukemia/lymphoma	Not impactful	Inadequate exposure assessment and study results do not add novel findings to a health effect that is well supported in the assessment
Xie et al. (2017)	General population Hong Kong case-control	Occupational history and industrial code, self-report, adults	Nasopharyngeal carcinoma incidence	Not impactful	Inadequate exposure assessment and study results do not add novel findings to a health effect that is well supported in the assessment

Rows for studies judged as "not i4mpactful" are shaded grey; unshaded rows highlight studies incorporated into the updated draft assessment.

F.3.7. Animal Studies of Respiratory Tract Cancer

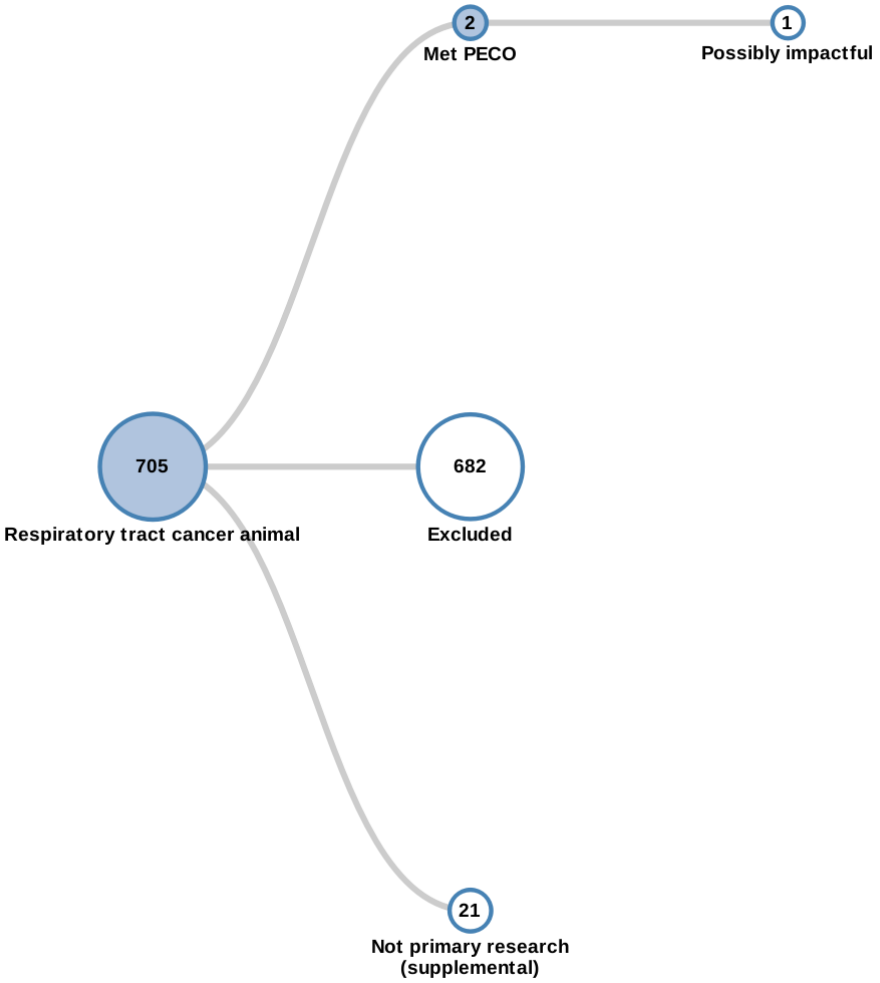


Figure F-7. Animal respiratory tract cancer literature tree (interactive version here).

A total of 705 citations were retrieved for the assessment of respiratory tract cancers in animals and 2 studies were PECO-relevant (Table F-9). Of these, one was deemed possibly impactful. This study, Morgan et al. (2017) was identified in the literature search update and included in the inventory, although it had been included in the 2017 draft Toxicological Review of Formaldehyde-Inhalation.

Table F-9. Animal studies of respiratory tract cancers

Reference	Study design	Exposure	Endpoints	Impact	Rationale
Animal Studies					
Morgan et al. (2017)	Mouse (Trp53 haploinsufficient), Male Subchronic (8 wk; 6 hr/d, 5 d/wk), then held for 32 wk	Paraformaldehyde 0, 7.5 or 15 ppm (0, 9.2, 18.5 mg/m^3) Inhalation	Blood was collected for hematology, and major tissues and gross lesions were collected for histopathology (nasal and LHP cancer, and respiratory lesions)	Possibly impactful	Already included in 2017 draft
Soffritti et al. (2016)	Rat (SD), both sexes Chronic (continuous exposure from 6–104 wks of age)	Unspecified test article 0, 50 ppm Oral (drinking water)	Carcinogenicity study (presumed to include evaluation of nasal/URT tumors)	Not impactful	Oral exposure; high levels; formalin (note: would be screened as excluded, but inventoried due to rarity of chronic exposure duration studies of cancer)

Rows for studies judged as "not impactful" are shaded grey; unshaded rows highlight studies incorporated into the updated draft assessment.

F.3.8. Animal Studies of Lymphohematopoietic Cancers

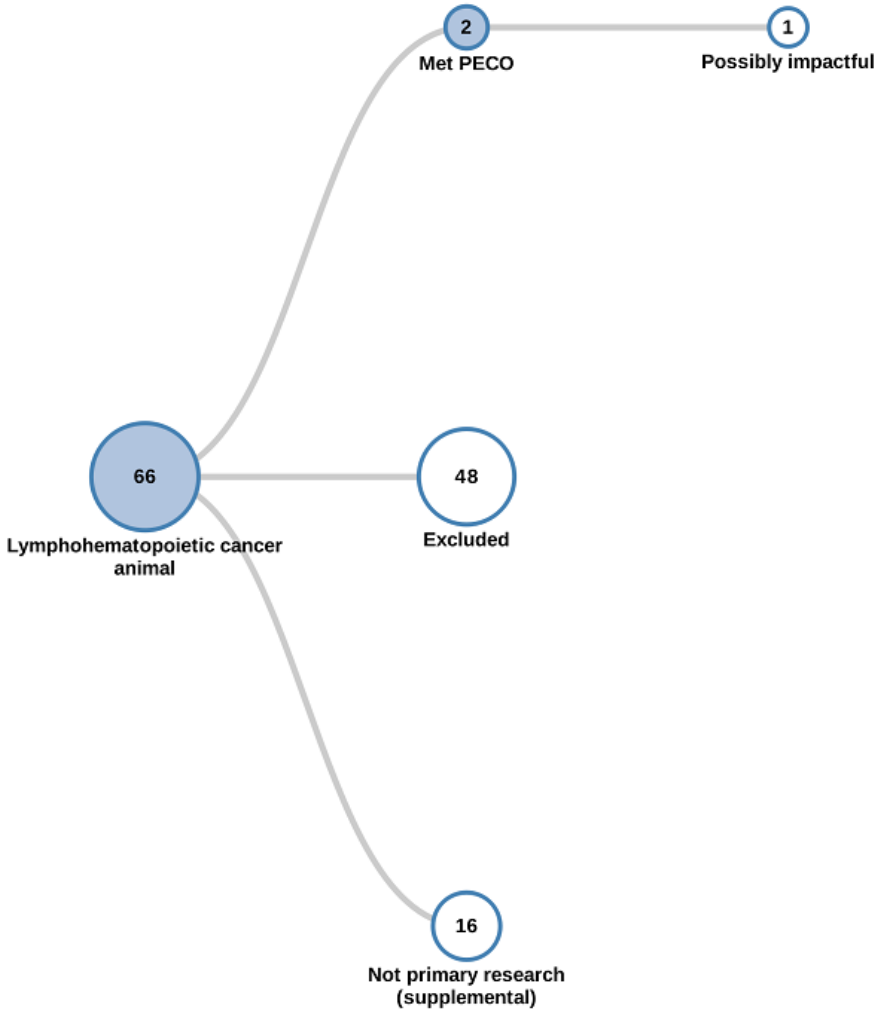


Figure F-8. Animal lymphohematopoietic cancer literature tree (interactive version here).

A total of 66 citations were retrieved for lymphohematopoietic cancers in animals and 2 studies were PECO-relevant (Table F-10). Of these, one was deemed possibly impactful. Morgan et al. (2017) was identified in the literature search update and included in the inventory, although it had been included in the 2017 draft Toxicological Review of Formaldehyde-Inhalation.

Table F-10. Animal studies of lymphohematopoietic cancer

Reference	Study design	Exposure	Endpoints	Impact	Rationale
Animal Studies					
Morgan et al. (2017)	Mouse (Trp53 haploinsufficient), Male Subchronic (8 wk; 6 hr/d, 5 d/wk), then held for 32 wk	Paraformaldehyde 0, 7.5 or 15 ppm (0, 9.2, 18.5 mg/m^3) Inhalation	All major tissues and gross lesions were collected for histopathology (including LHP tissues)	Possibly impactful	Already included in 2017 draft
Soffritti et al. (2016)	Rat (SD), both sexes Chronic (continuous exposure from 6–104 wks of age)	Unspecified test article 0, 50 ppm Oral (drinking water)	Carcinogenicity study (presumed to include evaluation of nasal/URT tumors)	Not impactful	Oral exposure; high levels; formalin (note: would be screened as excluded, but inventoried due to rarity of chronic exposure duration studies of cancer)

Rows for studies judged as "not impactful" are shaded grey; unshaded rows highlight studies incorporated into the updated draft assessment.

F.3.9. Mechanistic Studies of Inflammation and Immune-Related Responses

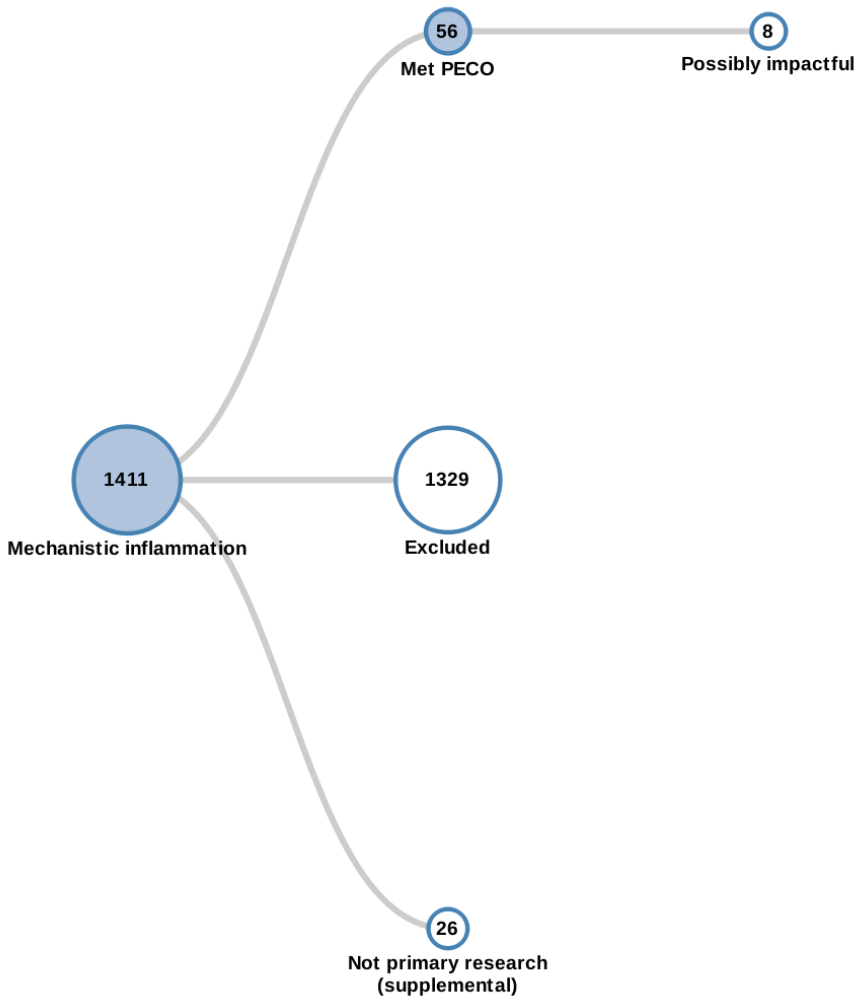


Figure F-9. Mechanistic inflammation and immune effects literature tree (interactive version here).

A total of 1,411 citations were retrieved for the assessment of mechanistic information on inflammation and immune responses (in the respiratory system or at systemic sites) and 56 studies were PECO-relevant (Table F-11). Of these, eight were deemed to be possibly impactful (note: one possibly impactful study is repeated under both the animal and in vitro/ex vivo sections). Morgan et al. (2017) was identified in the literature search update and included in the inventory table although it had been included in the 2017 draft Toxicological Review of Formaldehyde-Inhalation. In Vitro/ex Vivo designs and a study of endogenous formaldehyde biology also were included.

Table F-11. Mechanistic studies relating to respiratory or systemic inflammatory and immune responses

Reference	Study design	Exposure[a]	Mechanistic endpoints	Impact	Rationale
Human Studies					
Bassig et al. (2016)	Occupational Guangdong, China Cross-sectional	Air sampling Adult formaldehyde factory workers	WBC counts in blood, with subtype analyses of cells of both myeloid and lymphoid lineage (include CD4 T cell subtyping and cell activation markers)	Possibly impactful	PBL sub-population analyses and lineage studies are important endpoints
Costa et al. (2019)	Occupational Portugal Cross-sectional	Air sampling Adult anatomy-pathology laboratory workers	Lymphocyte counts, subpopulations analyses	Possibly impactful	PBL sub-population analyses and lineage studies are important endpoints
Augenreich et al. (2020)	Occupational Boone, North Carolina, USA Cohort	Air sampling Adult medical students in anatomy dissection rooms	Circulating markers of oxidative stress and inflammation; brachial artery dilation (arm), reactive hyperemia (leg), blood pressure/pulse/heart rate	Not impactful	ROS measures are not key endpoints
Bellisario et al. (2016)	Occupational Torino, Italy cross-sectional	Air sampling, adults, Female surgical nurses	Biomarkers of oxidative stress (urinary malondialdehyde and 15-F2t-isoprostane)	Not impactful	ROS markers are not key endpoints
Bruno et al. (2018)	Occupational Rome, Italy Cross-sectional	Air sampling Adult pathology laboratory workers	Counts of neutrophils, eosinophils, lymphocytes, macrophages, ratio of mucous-secreting cells and ciliated cells in the middle portion of the inferior turbinate	Not impactful	Cell counts (without sub-analyses) are not key endpoints
Ghelli et al. (2020)	Occupational Turin, Italy Cohort	Air sampling Adult (female) hospital workers	ROS measures in urine and inflammatory markers and cytokines in blood. Genotyped for CYP1A1, GSTT1, GSTM1, TNFa, and IL-6 polymorphisms	Not impactful	ROS and cytokine-related measures are not key endpoints
Isa et al. (2020a)	School-based Selangor, Malaysia Cross-sectional	Air sampling School children	Fractional exhaled nitric oxide (FeNO, an airway ROS/inflammation marker)	Not impactful	ROS markers are not key endpoints
Isa et al. (2020b)	School-based Hulu Langat, Selangor, Malaysia	Air sampling, children, Suburban and urban school children	Inflammatory cytokine markers in sputum; exhaled FeNO	Not impactful	ROS and cytokine-related measures

Reference	Study design	Exposure[a]	Mechanistic endpoints	Impact	Rationale
	Cross-sectional				are not key endpoints
Yon et al. (2019)	School-based Seongnam City, Korea Cohort	Air sampling School children	Serum formaldehyde-specific IgE; airway function; and exhaled FeNO	Not impactful	ROS and antibody-related measures are not key endpoints
Animal Studies					
Liu et al. (2017)	Mouse (ICR), male Subchronic (20 wk; 2 hr/d)	Unspecified test article 0, 1, 10 mg/m^3 Inhalation	Bone marrow cell MN; polychromatic erythrocytes (PCE)/normochromatic erythrocyte (NCE)ratio (immature/mature RBCs)	Possibly Impactful	Endpoints noted as important in draft; longer duration study is rare (note: presumed use of formalin limits interpretation)
Ma et al. (2020)	Mouse (Balb/c), male Subchronic (8 wk; 8 hr/d, 7 d/wk)	Formaldehyde in water (methanol free) 0, 2 mg/m^3 Inhalation	DNA damage (comet assay) in peripheral tissues (e.g., spleen; thymus); % of CD4+ T cells, CD8+ T cells, ratio of CD4+/CD8+ cells, and CD4 and CD8 cell phenotyping spleen weights, percentage of the DN (double negative), DP (double positive), CD4SP (single positive) and CD8SP cell populations in the isolated thymocytes, cytotoxicity in CD4SP and CD8SP cells, Runx (Runx 1,2,3, C), Runx1, Runx3, and ThPOK expression in the DP cells, ROS	Possibly impactful	Informative endpoints of immune cell health and function
Morgan et al. (2017)	Mouse (Trp53 haploinsufficient), Male Subchronic (8 wk; 6 hr/d, 5 d/wk), then held for 32 wk	Paraformaldehyde 0, 7.5 or 15 ppm (0, 9.23, 18.5 mg/m^3) Inhalation	Hematology	Possibly impactful	Already included in 2017 draft
Park et al. (2020)	Mouse (BALB/c), female Short-term (2 wk; 4 hr/d, 5 d/wk)	Fresh formaldehyde solution (methanol-free) 0, 1.38, 5.36 mg/m^3 Inhalation	Splenic cytokines, T cell populations and Th1/Th2 balance, differentiation markers	Possibly impactful	T cell subpopulation analyses are

Reference	Study design	Exposure[a]	Mechanistic endpoints	Impact	Rationale
					considered important
Zhao et al. (2020)	Mouse (Balb/c), male Short-term (2 wk; 8 hr/d, 5 d/wk)	Formalin 0, 3 mg/m^3 Inhalation	Burst-forming unit-erythroid (BFU-E), and colony-forming unit-granulocyte macrophage (CFU-GM) colonies in nose, lung, spleen, and bone marrow	Possibly impactful (POE tissues); Not impactful (systemic tissues)	Important endpoints (note: formalin; in vitro are of less concern for POE tissues)
Aydemir et al. (2017)	Rat (Wistar albino), both sexes Subchronic (6 wk; 8 hr/d, 5 d/wk)	Formalin 0, 6 ppm (0, 7.4 mg/m^3) Inhalation (note: i.p. not PECO relevant)	Blood DNA damage (comet assay) and ROS markers	Not impactful	Formalin; high level
Aydin et al. (2014)	Rat (Sprague-Dawley), male Short-term (4 wk)	Formalin 0, 5.27, 10.02 ppm (0, 6.48, 12.3 mg/m^3) Inhalation	Serum and lung total antioxidant and oxidant status, and oxidative stress index; serum glucose, protein, albumin, lipids, cholesterol, HDL, LDL, triglyceride, T protein; lung irisin levels and immunostaining	Not impactful	ROS and serum lipid-related measures are not key endpoints
Bernardini et al. (2020)	Mouse (Swiss), male Short-term (4 wk; 4 hr/d, 5 d/wk)	Unspecified test article 0, 0.5, 1, 5, 10 ppm (0, 0.62, 1.23, 6.15, 12.3 mg/m^3) Inhalation	Lung histopathology; BAL cell counts and inflammatory and ROS markers; global methylation in blood and bone marrow	Not impactful	Unknown test article; not key endpoints
Cheng et al. (2016)	Mouse (Kunming), male Short-term (3 or 7 d; continuous)	Formalin 0, 0.08, 0.8 mg/m^3 Inhalation	Serum CD4+, CD8+, and CD4/CD8 T cell counts	Not impactful	Formalin
Abreu et al. (2016)	Mouse (C57BL/6), female Acute (single exposure, assessed 8 hr later)	Unspecified test article 0, 0.2, 1, 3 ppm (0, 0.25, 1.23, 3.69 mg/m^3) Inhalation	Lung mechanics and morphology, inflammatory cell counts and cytokines, and ROS markers	Not impactful	Unknown test article; acute
da Silva et al. (2015)	Rat (Wistar), male	Unspecified test article 0, 1 %	BAL cell counts (WBCs, Mono., Lympho., Neutro., Eosin.), cytokines, and	Not impactful	Unknown test article; high levels

Reference	Study design	Exposure[a]	Mechanistic endpoints	Impact	Rationale
	Short-term (3 d; 90-min/d)	Inhalation	myeloperoxidase activity (inflammation); lung morphometrics, microvascular permeability, and mRNA levels		
Duan et al. (2018)	Mouse (BALB/c), male Short-term (18 d; 5 hr/d)	Formalin 0, 1 mg/m^3 Inhalation	Pulmonary eosinophil cationic protein (histopathology), ROS markers, nuclear factor kappa B activation, and cytokine and growth factor levels	Not impactful	Formalin; no saline plus formaldehyde control group
Duan et al. (2020)	Mouse (Balb/c), male Short-term (21 d; 6 hr/d)	Formalin 0, 0.5 mg/m^3 Inhalation	Airway IgE, cytokines and inflammatory factors, Th1/Th2 balance, mucus secretion, histopathology, and lung function	Not impactful	Formalin; not key endpoints
Ge et al. (2020a)	Mouse (Balb/c), male Short-term (2 wk; 8 hr/d, 5 d/wk)	Formalin 0,0.5, 3 mg/m^3 Inhalation	CBC; Myeloid progenitor cell (BFU-E and CFU-GM) colony counts and cytokines; circulating ROS and cytokine markers; bone marrow histology, ROS, and gene expression of cell cycle and DNA damage markers	Not impactful	Formalin
Han et al. (2016)	Rat (Sprague-Dawley), male Subchronic (6 wk; 2 hr/d, 5 d/wk beginning at PND3	Paraformaldehyde 0, 0.83, 1.16 ppm (0, 1.02, 1.43 mg/m^3) Inhalation	Serum IgE, thymus Th1 and Th2 cytokines, body weight	Not impactful	Nonspecific antibodies and cytokines are not key endpoints
Jin et al. (2021)	Mouse (C57BL/6J), both sexes Short-term (4 d; 6 hr/d)	Unspecified test article 0, 5 ppm (0, 6.15 mg/m^3) Inhalation	Respiratory parameters (e.g., rate) during exposure; serum lipids; serum cell counts and soluble factors (CBC)	Not impactful	Unknown test article; not key endpoints
Kang et al. (2018)	Mouse (BALB/c), male Short-term (18 d; 5 hr/d)	Formalin 0, 1 mg/m^3 Inhalation	Serum IgE, IgG; airway hyperreactivity, ROS markers, nuclear factor kappa B and MAPK activation; cytokine levels, and mast cell degranulation	Not impactful	Formalin; not key endpoints
Leal et al. (2018)	Mouse (C57BL6), male Short-term (2 wk; 1 hr/d, 5 d/wk)	Unspecified test article 0, 0.92 mg/m^3 Inhalation	Lung cytokines and elasticity measures	Not impactful	Unknown test article; not key endpoints
Li et al. (2017)	Mouse (Balb/c or C57BL/6), male	Formalin 0, 0.5, 3 mg/m^3 Inhalation	Bronchial responsiveness (to methacholine), BAL cytokines and cell counts (total, eosin.,	Not impactful	Formalin; not key endpoints

Reference	Study design	Exposure[a]	Mechanistic endpoints	Impact	Rationale
	Short-term (25 d; 6 hr/d)		lympho., neutro.); Serum OVA-specific IgE, IgG1, and IgG2a		
Lima et al. (2015)	Rat (Fischer), male Short-term (5 d; 20-min x3/d)	Unspecified test article 0, 1, 5, 10 % Inhalation	Trachea histology and morphometric analyses, including mucus production, glycogen, ROS markers, and inflammatory cell counts.	Not impactful	Unknown test article; high levels
Liu et al. (2018b)	Rat (Sprague Dawley), male Short-term (4 wk; 8 hr/d)	Formalin 0, 0.5, 5, 10 mg/m^3 Inhalation	Lung autophagy, histopathology and BAL cytokines	Not impactful	Formalin; not key endpoints
Macedo et al. (2016b)	Rat (Wistar), male Short-term (3 d; 90-min/d)	Formalin 0, 1 % Inhalation	BAL ROS markers and cellular oxidative burst; lung tissue antioxidant enzyme measures	Not impactful	Formalin; high levels
Murta et al. (2016)	Rat (Fischer), male Short-term (5 d; 20-min × 3/d)	unspecified 0, 1, 5, 10 %, inhalation	BALF cell counts (WBCs, macrophages, lymphocytes, neutrophils, eosinophils), inflammatory and ROS markers, and neutrophil ROS production Lung tissue inflammatory markers, H&E staining and morphometry	Not impactful	Unknown test article; high levels
Payani et al. (2019)	Rat (Wistar, albino), male Short-term (21 d; 1 hr/d)	Unspecified test article 0, 40 % Inhalation	Lung ROS markers	Not impactful	Unknown test article; high levels
Sapmaz et al. (2015)	Rat (Sprague-Dawley), male Short-term (4 wk; 8 hr/d)	Paraformaldehyde 0, 5, 10 ppm (0, 6.15, 12.3 mg/m^3) Inhalation	Serum total IgA, IgM, IgG, complement C3	Not impactful	Nonspecific antibody-related measures are not key endpoints
Sholapuri et al. (2020)	Rat (Wistar), male Short-term (21 d; 1 hr/d)	Formalin 0, 40 % Inhalation	Hematology parameters (CBC); BAL histamine; lung histology	Not impactful	Formalin; high levels
Song et al. (2017)	Mouse (Balb/c), male Short-term (25 d)	Formalin 0, 2.44 ppm (0, 3 mg/m^3) Inhalation	Serum levels of cytokines, neuropeptides, ROS, and IgE; leukocyte counts and cellular antioxidant levels.	Not impactful	Formalin; No formaldehyde-only control (without OVA);
Wei et al. (2017b)	Mouse (BALB/c), male	Formalin 0, 3 mg/m^3	Complete blood cell count; bone marrow - myeloid progenitor formation assay, ROS	Not impactful	Formalin; short-term (otherwise

Reference	Study design	Exposure[a]	Mechanistic endpoints	Impact	Rationale
	Short-term (2 wk; 8 hr/d, 5 d/wk)	Inhalation	assay, IL-3 and GM-CSF ELISA, systemic toxicity, bone marrow cellularity, apoptosis assay		important endpoints)
Wei et al. (2017a)	Mouse (BALB/c), male Short-term (2 wk; 5 d/wk), followed by 7 d recovery	Formalin 0, 3 mg/m^3 Inhalation	Complete blood cell count, bone marrow histopathology, myeloid progenitor colony-forming cell assay, ROS and cytokine measures, and DNA-protein crosslinks	Not impactful	Formalin; short-term (otherwise important endpoints)
Wen et al. (2016)	Mouse (Balb/c), male Short-term (2 wk; 8 hr/d, 5 d/wk)	Formalin 0, 3 mg/m^3 Inhalation	Cell counts (WBCs, lymphocytes, monocytes, neutrophils, RBCs, platelets); serum antibody (total) level; ROS markers; PBL proliferation; serum hemagglutination titer and delayed-type hypersensitivity (both after sheep RBC injection)	Not impactful	Formalin (limits interpretability of systemic effects)
Wu et al. (2020)	Mouse (Balb/C), male Short-term (21 d; 5 hr/d)	Formalin 0, 0.8 mg/m^3 Inhalation	Pulmonary function; lung histopathology; airway hyperresponsiveness; lung IgE and cytokine (including Th1/Th2) levels	Not impactful	Formalin; not key endpoints
Zhang et al. (2018b)	Mouse (Balb/c), male Short-term (7, 14, or 28 d, 2 4hr/d for constant and 12 hr/d for intermittent)	Unspecified test article 0, 0.8 (intermittent) or 0, 0.4 (constant) ppm (0, 0.49, or 0.98 mg/m^3) Inhalation	BAL cell counts (total, eosin., neutro., lympho.); lung tissue ROS markers, histology, and cytokine and inflammatory marker immunohistochemistry	Not impactful	Unknown test article; not key endpoints
In Vitro/Ex Vivo Studies					
Zhao et al. (2020)	Mouse (Balb/c), male Ex vivo primary lung and nose cells (systemic cells not PECO-relevant) Acute (1 hr)	Formalin 0, 50, 100, 200, 400 µM In media	Burst-forming unit-erythroid (BFU-E), and colony-forming unit-granulocyte macrophage (CFU-GM) colonies	Possibly impactful	Important endpoints (note: formalin; in vitro are of less concern for POE tissues)
An et al. (2019)	Human immortalized bronchial epithelial cells (in vitro experiments in LHP-relevant cells were excluded)	Unspecified test article 0, 20, 40, 60, 80, 100, 120 µM In media	Cell proliferation, ROS production, and markers of cell division/proliferation and ROS	Not impactful	Unknown test article; in vitro; acute

Reference	Study design	Exposure[a]	Mechanistic endpoints	Impact	Rationale
	Acute (2 hr)				
Arslan-Acaroz and Bayşu-Sozbilir (2020)	Human immortalized lung epithelial cells Acute (4 hr)	Unspecified test article 0, 100 µM, In media	Cell viability and ROS markers	Not impactful	Unknown test article; in vitro; acute
Boncler et al. (2019)	Human immortalized lung epithelial cells (other in vitro experiments in this study excluded as not PECO relevant) Acute (24 hr)	Unspecified test article 0, 63, 126, 378, 504, 630 µmol/L In media	Cell viability and mitochondrial membrane potential	Not impactful	Unknown test article; in vitro; acute
Cui et al. (2016)	Human immortalized lung cells or Mouse (Balb/c) nasal instillation Acute up to 48 hr	Unspecified test article 0, 200 µM In media or instilled	Cell signaling and gene expression, ROS, and cellular currents	Not impactful	Unknown test article; acute
Gostner et al. (2016)	Human immortalized, lung epithelial cells Short-term (3 d)	Unspecified test article 0, 0.1, 0.5 ppm (0, 0.12, 0.62 mg/m^3) Gaseous exposure at the air:liquid interface	Cell viability; gene expression	Not impactful	Unknown test article; not key endpoints
Jude et al. (2016)	Human primary airway smooth muscle (HASM) cells Acute (1 hr, assessed at 24 hr)	Formalin0, 0.2, 0.8, 2 ppm (0, 0.25, 0.98, 2.46 mg/m^3) Vapor delivered to cells	Agonist-induced calcium mobilization, cytotoxicity, ROS markers and cytokines in co-cultures; cabachol-induced airway narrowing in slices	Not impactful	Formalin; in vitro; acute
Kim et al. (2018)	Human immortalized endometrial adeno-carcinoma cells Short-term (6 d) [Note: study included due to use of this cell line to examine mechanisms associated with	Unspecified test article 10^{-11} to 10^{-3} M In media	ROS production, protein expression of markers associated with cell transformation and proliferation	Not impactful	Unknown test article; in vitro

Reference	Study design	Exposure[a]	Mechanistic endpoints	Impact	Rationale
	epithelial cell-cell interactions]				
Li et al. (2008)	Human immortalized tracheal epithelial cells Acute (4 or 24 hr)	Unspecified test article 0, 20, 50, 100, 200 µM In media	Cell viability and expression of MAPK-responsive genes	Not impactful	Unknown test article; in vitro; acute
Liu et al. (2019)	Human immortalized bronchial epithelial cells Acute (24 hr)	Unspecified test article 0, 40, 80, 160 µmol/L In media	Apoptosis, PI3K-Akt pathway signaling markers	Not impactful	Unknown test article; in vitro; acute
Mi et al. (2019)	Human pulmonary alveolar epithelial cells in artificial airway Acute (2, 4, or 6 hr)	Unspecified test article 0.025 and 40 µM (0.025 µM = ~0.3 ppm) Nitrogen carrier-mediated delivery directly into cells	ROS and cytokine markers	Not impactful	Unknown test article; acute
Nazarparvar-Noshadi et al. (2020)	Human immortalized lung epithelial cells Acute/short-term (24, 48, and 72 hr)	Unspecified test article 0, 25, 50, 100, 150, 200, 300 µM In media	Cellular viability and DNA damage markers	Not impactful	Unknown test article; in vitro
Vitoux et al. (2018)	Human immortalized conjunctival epithelial cells Acute (15–30 min, assess at 1 or 24 hr)	Formalin 0, 100, 1,200 µg/m^3 Airflow over cells	Expression of inflammatory cytokines	Not impactful	Formalin; in vitro; acute
Zhang et al. (2019)	Human immortalized lung bronchial cells Acute (3, 6, 12, or 24 hr)	Formalin 0, 5, 10, 20, 40, 80 µmol/L In media	ROS and cytotoxicity markers m	Not impactful	Formalin; in vitro; acute
Zhang et al. (2020b)	Human Immortalized bronchial epithelial cells	Formalin 0, 10, 40, 80 µM 24 h	DNA damage - comet assay; apoptosis; mitochondria-mediated apoptosis; reactive oxygen species levels	Not impactful	Formalin; in vitro; non-critical endpoints
Models, Endogenous Formaldehyde, or Other Studies					
Dingler et al. (2020)	Mouse (C57BL/6 background), ALDH2	No formaldehyde inhalation exposures	Genotoxicity in peripheral blood cells and bone marrow (MN assay, SCE); bone	Possibly impactful	Serves as included reference study for

Reference	Study design	Exposure[a]	Mechanistic endpoints	Impact	Rationale
	and ALDH5 WT, single, and double KO, both sexes (note: also includes primary cultures of human PBLs, fibroblasts, and buccal cells not deemed PECO-relevant)	(note: included since it evaluates essentiality of formaldehyde detoxification processes in normal function)	marrow stem cell and progenitor cell quantification, lineage characterization, and B cell maturation; thymic development and cell lineage characterization; complete blood cell count, cell cycle profiling		discussion of potential sources of susceptibility relating to formaldehyde detoxification; hematopoietic health and cell production from bone marrow is important endpoint

Abbreviations: WBC = white blood cell; ROS = reactive oxygen species; BAL = bronchoalveolar lavage (F = fluid); RBC = red blood cell; PBL = peripheral blood leukocyte; CBC = complete blood cell (count).

Rows for studies judged as "not impactful" are shaded grey; unshaded rows highlight studies incorporated into the updated draft assessment.

[a] Use of methanol-stabilized formalin was inferred in some studies based on study-specific description (e.g., 37% stock solution).

F.3.10. Mechanistic Studies of Respiratory Tract Cancer, Focusing on Genotoxicity

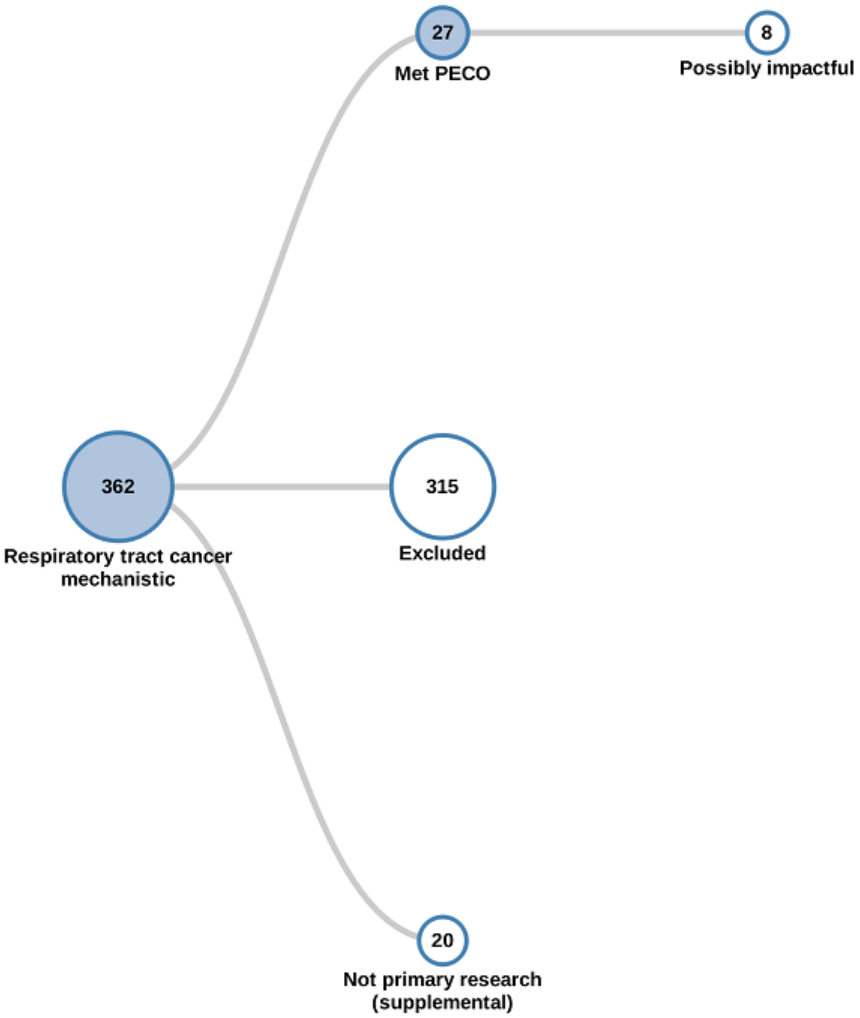


Figure F-10. Mechanistic respiratory tract cancer literature tree (interactive version here).

A total of 362 citations were retrieved for the assessment of mechanistic information informing respiratory tract cancers, focusing on genotoxicity, and 27 studies were PECO-relevant. Of these, eight studies were deemed to be possibly impactful (note: one possibly impactful study is repeated under both the animal and in vitro/ex vivo sections). Table F-12 summarizes studies of formaldehyde exposure in humans and animals, as well as in vitro or ex vivo experiments. Several studies relevant to endogenous formaldehyde, pharmacokinetic modeling and dosimetry also were included.

Table F-12. Mechanistic studies relating to respiratory tract cancers, focusing on genotoxicity

Reference	Study design	Exposure[a]	Mechanistic endpoints	Impact	Rationale
Human Studies					
Aglan and Mansour (2018)	Occupational Cairo, Egypt Cross-sectional	Air sampling Adult hairstylists	Buccal cell MN frequency	Possibly impactful	Specific markers; exposures similar to important studies in draft
Costa et al. (2019)	Occupational Portugal Cross-sectional	Air sampling Adult anatomy-pathology laboratory workers	Buccal cell MN and nuclear budding, genotype analysis of selected polymorphisms	Possibly impactful	Specific markers; exposures similar to important studies in draft
Peteffi et al. (2015)	Occupational Rio Grande do Sul, Brazil Cross-sectional	Air sampling Adult furniture workers	Micronucleus (MN) assay in buccal cells: nuclear buds, binucleated cells, Karyorrhexis	Possibly impactful	Specific markers; exposures similar to important studies in draft
Bono et al. (2016)	Occupational Piedmont region, Italy Cross-sectional	Air sampling Adult plastic laminate workers	Malondialdehyde DNA adducts in swabbed nasal epithelial cells	Not impactful	Adducts may or may not lead to more robust markers
Bruno et al. (2018)	Occupational Rome, Italy Cross-sectional	Air sampling Adult pathology laboratory workers	Counts of multinucleated ciliated cells, Karyorrhexis, Hyperchromatic SNS from middle portion of the inferior turbinate	Not impactful	Nuclear abnormalities are non- specific markers
Animal Studies					
Leng et al. (2019)	Rat (Fischer 344), male Short-term (28 d; 6 hr/d)	Deuterated formaldehyde (no methanol) 0, 1, 30, 300 ppb (1.23, 36.9, 369 mg/m^3) [$^{13}CD_2$]-HCHO Inhalation	DNA adducts in nose, lung (and other tissues)	Possibly impactful	Endpoints important to dosimetry; low exposure levels
Zhao et al. (2020)	Mouse (BALB/c), male Short-term (2 wk; 8 hr/d, 5 d/wk)	Formalin 0, 3 mg/m^3 Inhalation	Burst-forming unit-erythroid (BFU-E), and colony-forming unit-granulocyte macrophage (CFU-GM) colonies from nose and lung	Possibly impactful	Impactful endpoints (Note: formalin, but less of a concern in POE)

Reference	Study design	Exposure[a]	Mechanistic endpoints	Impact	Rationale
Bernardini et al. (2020)	Mouse (Swiss), male Short-term (4 wk; 4 hr/d, 5 d/wk)	unspecified test article 0, 0.5, 1, 5, 10 ppm (0, 0.62, 1.23, 6.15, 12.3 mg/m^3) Inhalation	MN, comet assay, and global methylation in lung	Not impactful	Unknown test article; no specific URT measures
Edrissi et al. (2017)	Rat (F344), male Short-term (7, 14, 21, or 28 d; 6 hr/d)	[^{13}C]-labeled formaldehyde 0, 2 ppm (0, 2.46 mg/m^3) Inhalation	FA-lysine adducts in nasal epithelium, lung, and trachea	Not impactful	Adducts may or may not lead to more robust markers
In vitro/Ex vivo Studies					
Zhao et al. (2020)	Mouse (BALB/c), male Ex vivo primary lung and nose cells Acute (1 hr)	Formalin 0, 50, 100, 200, 400 µM In media	Burst-forming unit-erythroid (BFU-E), and colony-forming unit-granulocyte macrophage (CFU-GM) colonies	Possibly impactful	Important endpoints (note: formalin; in vitro)
Anandarajan et al. (2020)	Yeast (*Schizosaccharomyces pombe*), deletion strains Short-term (3-5 d)	Formalin 0, 0.2, 0.5, 1.25, 1.5, 1.75 mM (Note: included due to conserved DNA repair pathways between yeast and humans, and potential relevance to human susceptibility)	Toxicogenomic profiling of pathways relating to formaldehyde detoxification and DNA repair–including homologous recombination and nucleotide excision repair	Not impactful	Yeast; formalin; high dose
Chen et al. (2017)	Human immortalized bronchial epithelial cells Acute (up to 6 hr)	Unspecified test article 0, 0.5 mM In media	Inhibition of chromatin assembly, formaldehyde-histone adducts, gene expression	Not impactful	Unknown test article; in vitro; non-critical endpoints
Gonzalez-Rivera et al. (2020)	Human immortalized bronchial epithelial cells Acute (2 hr)	Paraformaldehyde 0, 1 ppm (0, 1.23 mg/m^3) In vitro gaseous exposure	Cell phenotypic alterations; DNA damage	Not impactful	In vitro; non-critical endpoints
Juarez et al. (2018)	Human immortalized, osteosarcoma, fibroblast, or epithelial colorectal adenocarcinoma cells	Unspecified test article 0, 20, 40, 60, 80, 100 µM In media	genomic analysis (Note: included due to analyses across multiple cell lines which might reflect genomic	Not impactful	In vitro; indirect measure; no cell lines specific to URT

Reference	Study design	Exposure[a]	Mechanistic endpoints	Impact	Rationale
	Short-term (5 d; continuous)		signatures relevant to exposure of URT cells)		
Kang et al. (2016)	Yeast (*Saccharomyces cerevisiae),* deletion strains 5 or 15 generations of exposure	Unspecified test article 0, 0.15, 0.3, 0.6 mM (Note: included due to conserved DNA repair pathways between yeast and humans, and potential relevance to human susceptibility)	Toxicogenomic profiling of pathways relating to RNA stability and DNA repair– including homologous recombination, single strand annealing, and post-replication repair	Not impactful	Yeast; Unknown test article; high dose
Nazarparvar-Noshadi et al. (2020)	Human immortalized lung epithelial cells Acute (24 hr; note: cytotoxicity up to 72 hr)	Unspecified test article 0, 25, 50, 100, 150, 200, and 300 µM In media	DNA damage (DNA ladder) and cytotoxicity/ apoptosis	Not impactful	Unknown test article; in vitro; non-critical endpoints
Zhang et al. (2018a)	Human immortalized alveolar basal epithelial cells Acute (24 hr)	Freshly prepared formaldehyde solution 25 to 1,500 µM In media	DNA damage; chromosome damage; micronucleus frequency; cytotoxicity	Not impactful	In vitro (many in vivo studies exist)
Zhang et al. (2020a)	Human immortalized bronchial epithelial cells Acute (3, 6, 12, 24 hr)	Formalin 0, 5, 10, 20, 40, 80 µM In media	DNA strand breaks; chromosome damage; DNA repair, ROS, and cell cycle markers	Not impactful	Formalin; in vitro; non-critical endpoints
Zhang et al. (2020b)	Human Immortalized bronchial epithelial cells Acute (24 hr)	Formalin 0, 10, 40, 80 µM In media	DNA damage - comet assay; apoptosis; mitochondria-mediated apoptosis; reactive oxygen species levels	Not impactful	Formalin; in vitro; non-critical endpoints
Modeling, Endogenous Formaldehyde, and Other Studies					
Campbell Jr et al. (2020)	Updated pharmacokinetic model developed here for formaldehyde dG adducts based on the previously developed models for formaldehyde DPX (Andersen et al., 2010); Conolly et al. (2000).			Possibly impactful	Model potentially important to modeling dosimetry (Note: discussed with regard to toxicokinetics, Section 1.1.3, and cancer dose-response, Section 2.2.1, not MOA analysis, Section 1.2.5)

Reference	Study design	Exposure[a]	Mechanistic endpoints	Impact	Rationale
Corley et al. (2015)	Excerpt from abstract: extended airway computational fluid dynamic (CFD) models of the rat and human were coupled with airway region-specific physiologically based pharmacokinetic (PBPK) tissue models to describe the kinetics of formaldehyde. Simulations of aldehyde no-observed-adverse-effect levels for nasal toxicity in the rat were conducted until breath-by-breath tissue concentration profiles reached steady state. Human oral breathing simulations were conducted using representative aldehyde yields from cigarette smoke.			Possibly impactful	Model potentially important to modeling dosimetry (Note: discussed with regard to toxicokinetics, Section 1.1.3, and cancer dose-response, Section 2.2.1, not MOA analysis, Section 1.2.5)
Miller et al. (2017)	BBDR: Previously a computational fluid dynamics model was combined with a 2-stage clonal growth model to develop a MOA-based DR model. This paper reports changes that reflect a better understanding of populations of cells at risk of carcinogenic transformation in the pharynx, larynx and respiratory bronchiolar portions of the human respiratory tract and inclusion of basal cells in the pool of cells at risk.			Possibly impactful	Model potentially important to modeling dosimetry (Note: discussed with regard to cancer dose-response, Section 2.2.1, not MOA analysis, Section 1.2.5)
Burgos-Barragan et al. (2017)	Mouse (C57BL/6 × 129SV hybrid background), WT or KO in ALDH2, FANCD2, or both (note: also included in vitro evaluations in human, chicken, and mouse cells)	No formaldehyde inhalation exposures (note: included since it evaluates essentiality of formaldehyde detoxification processes in normal function)	Genotoxicity (DNA damage response markers) in vitro and in vivo (various tissues) when formaldehyde detoxification pathways are disrupted	Not impactful	Included as reference study for discussion of potential sources of susceptibility relating to formaldehyde detoxification
Starr and Swenberg (2016)	Update to prior non-primary research perspectives on how to calculate cancer risk			Not impactful	Included due to discussion in 2017 draft, but non-primary research
Yang et al. (2020)	Excerpt from abstract: the deposition rates from the linear regressions of CH_2O, CH_5N, C_2H_6O, $C_2H_4O_2$, C_3H_8O, C_6H_6, C_7H_8, C_8H_8, and C_8H_{10} of 120 healthy volunteers were obtained with significantly different from the respective calculated deposition rates. In order to explore the effects of the breathing models and sampling time on the deposition rates of VOCs, volunteers were first asked to breathe successively with nasal-in-nasal-out, oral-in-nasal-out, and oral-in-oral-out breathing models before and after three meals for 3 d. In order to further validate the results, the deposition rates of the selected VOCs were calculated in 120 healthy volunteers using nasal-in-oral-out breathing model for unlimited time after the conventional lung function examination.			Not impactful	Not impactful to dosimetry modeling in the assessment (note: briefly discussed in the assessment as consistent with prior observations)
Yoo and Ito (2018a)	BBDR: PBPK-computational fluid dynamics hybrid analysis was integrated into the computer simulated person-based numerical simulation to estimate inhalation exposure and respiratory tissue dosimetry with the unsteady breathing cycle model.			Not impactful	Not impactful to dosimetry modeling in the assessment (see below)

Reference	Study design	Exposure[a]	Mechanistic endpoints	Impact	Rationale
Yoo and Ito (2018b)	Excerpt from abstract: In this study, a CSP integrated with a virtual airway was developed and used to estimate inhalation exposure in an indoor environment. The virtual airway is a numerical respiratory tract model for CFD simulation that reproduces detailed geometry from the nasal/oral cavity to the bronchial tubes by way of the trachea. Physiologically based pharmacokinetic (PBPK)-CFD hybrid analysis is also integrated into the CSP. Through the coupled simulation of PBPK-CFD-CSP analysis, inhalation exposure under steady state conditions where formaldehyde was emitted from floor material was analyzed and respiratory tissue doses and their distributions of inhaled contaminants are discussed quantitatively.			Not impactful	Not impactful to dosimetry modeling in the assessment [these studies by Yoo and Ito (2018a, b), extended the Corley et al. (2015) modeling by superposing on it the dynamics of formaldehyde exterior to the respiratory tract (i.e. within the room and surrounding the nose and mouth). As such they do not provide additional information of relevance to the assessment beyond that discussed in the context of Corley et al. (2015)]

Rows for studies judged as "not impactful" are shaded grey; unshaded rows highlight studies incorporated into the updated draft assessment.
Abbreviations: MN = micronucleus (assay); ROS = reactive oxygen species; BBDR = biologically based dose-response (model).
[a] Use of methanol-stabilized formalin was inferred in some studies based on study-specific description (e.g., 37% stock solution).

F.3.11. Mechanistic Studies of Lymphohematopoietic Cancer, Focusing on Genotoxicity

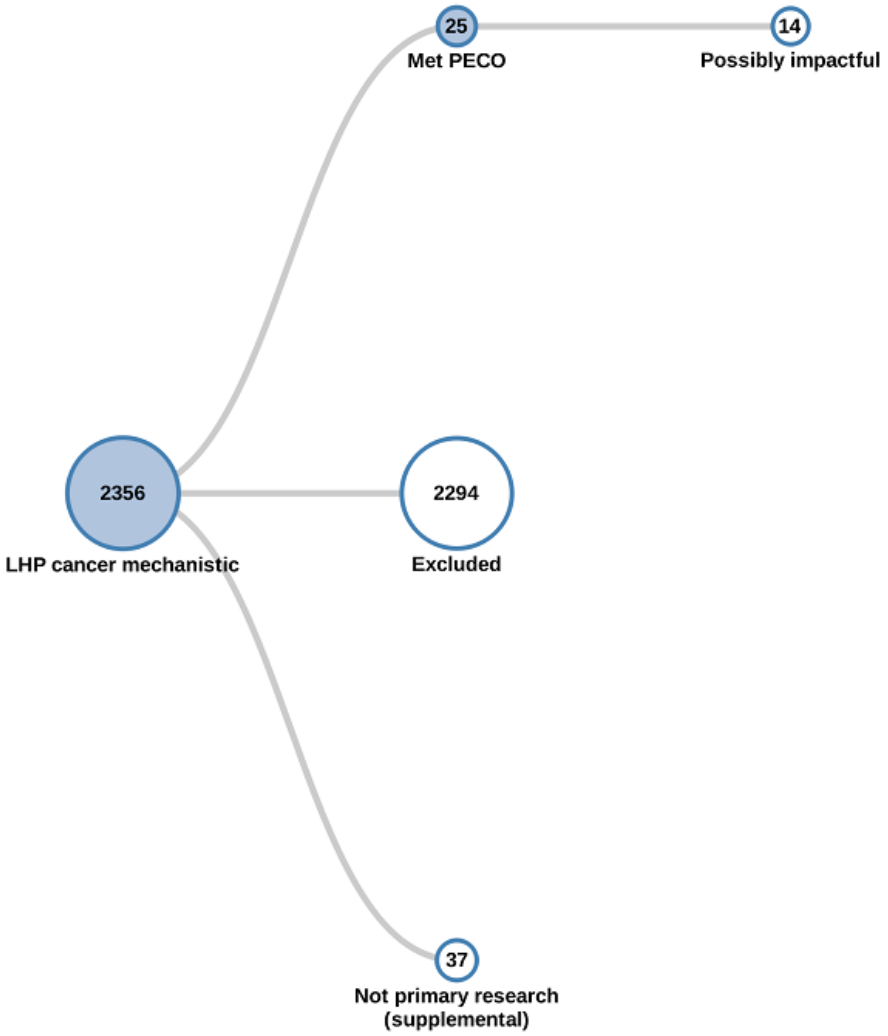


Figure F-11. Mechanistic lymphohematopoietic cancer literature tree (interactive version here).

A total of 2,356 citations were retrieved for the assessment of mechanistic information informing lymphohematopoietic cancers, focusing on genotoxicity, and 25 studies were PECO-relevant (Table F-13). Of these, 14 studies were deemed to be possibly impactful. Studies relevant to pharmacokinetic modeling or dosimetry also were included. Mundt et al. (2017) was identified in the literature search update and included in the inventory table although it already had been included in the 2017 draft Toxicological Review of Formaldehyde-Inhalation.

Table F-13. Mechanistic studies relating to lymphohematopoietic cancers, focusing on genotoxicity

Reference	Study design	Exposure[a]	Mechanistic endpoints	Impact	Rationale
Human Studies					
Aglan and Mansour (2018)	Occupational Cairo, Egypt Cross-sectional	Air sampling Adult hairstylists	PBL MN	Possibly impactful	Specific markers; exposures similar to important studies in draft
Bassig et al. (2016)	Occupational Guangdong, China Cross-sectional,	Air sampling Adult formaldehyde factory workers	Frequency of monosomy 7 in isolated CFU-GM cells	Possibly impactful	Specific markers; exposures similar to important studies in draft
Costa et al. (2015)	Occupational Northern and Central Portugal Cross-sectional	Air sampling Adult pathology workers	Chromosomal aberrations, comet assay, genotype analysis in blood cells	Possibly impactful	Specific markers; exposures similar to important studies in draft
Costa et al. (2019)	Occupational Portugal Cross-sectional	Air sampling Adult anatomy-pathology laboratory workers	PBL MN and sister chromatid exchange; T-cell receptor mutations; genotype analysis of select polymorphisms	Possibly impactful	Specific markers; exposures similar to important studies in draft
Mundt et al. (2017)	Occupational China Cross-sectional	Additional analysis of Zhang (2010) results Adult factory workers	Monosomy of chromosome 7 and 8, complete blood count	Possibly impactful	Already identified in 2017 draft
Peteffi et al. (2015)	Occupational Rio Grande do Sul, Brazil Cross-sectional	Air sampling Adult furniture workers	Comet assay in PBLs [cell migration, frequency of damaged cells, damage index]	Possibly impactful	Markers of DNA damage; exposures similar to important studies in draft
Wang et al. (2019)	Occupational Shanghai, China Cross-sectional	Air sampling Adult factory workers	Cytokinesis-blocked MN assay in PBLs	Possibly impactful	Specific markers; exposures similar to important studies in draft
Zendehdel et al. (2017)	Occupational Tehran City, Iran Cross-sectional	Air sampling Adult melamine workers	Comet assay [tail moment, Olive moment in PBLs]	Possibly impactful	Markers of DNA damage; exposures similar to important studies in draft

Reference	Study design	Exposure[a]	Mechanistic endpoints	Impact	Rationale
Barbosa et al. (2019)	Occupational Porto Alegre, Brazil Cross-sectional	Air sampling Adult beauty salon workers	Global DNA methylation (%) in PBLs	Not impactful	Not specific to genotoxicity, so less important endpoint
Zendehdel et al. (2018)	Occupational Tehran, Iran Cross-sectional	Air sampling Adult melamine workers	DNA damage (comet assay) in PBLs	Not impactful	Related to Zendehdel et al. (2017), no additional results.
Animal Studies					
Leng et al. (2019)	Rat (Fischer 344), male Short-term (28 d; 6 hr/d)	Deuterated formaldehyde (no methanol) 0, 1, 30, 300 ppb (0, 1.23, 36.9, 369 µg/m^3) Inhalation	DNA adducts in blood, bone marrow (and other tissues)	Possibly impactful	Endpoints important to dosimetry; low exposure levels
Liu et al. (2017)	Mouse (ICR), male 20 wk (2 hr/d)	Unspecified test article 0, 1, 10 mg/m^3 Inhalation	Bone marrow cell MN; polychromatic erythrocytes (PCE)/normochromatic erythrocyte (NCE) ratio (immature/mature RBCs)	Possibly Impactful	Endpoints noted as important in draft; longer duration study (note: presumed use of formalin limits interpretation)
Ma et al. (2020)	Mouse (Balb/c), male Subchronic (8 wk; 8 hr/d, 7 d/wk)	Formaldehyde in water (methanol free) 0, 2 mg/m^3 Inhalation	DNA damage (comet assay) in peripheral tissues (e.g., spleen; thymus); % of CD4+ T cells, CD8+ T cells, ratio of CD4+/CD8+ cells, and CD4 and CD8 cell phenotyping	Possibly impactful	Informative endpoints of immune cell health and function
Aydemir et al. (2017)	Rat (Wistar albino), both sexes Subchronic (6 wk; 8 hr/d, 5 d/wk)	Formalin 0, 6 ppm (0, 7.38 mg/m^3) Inhalation (note: i.p. deemed not PECO relevant)	DNA damage (comet assay) and ROS markers in peripheral blood	Not impactful	Formalin; high level
Bernardini et al. (2020)	Mouse (Swiss), male Short-term (4 wk; 4 hr/d, 5 d/wk)	unspecified test article 0, 0.5, 1, 5, 10 ppm (0, 0.62, 1.23, 6.15, 12.3 mg/m^3) Inhalation	MN, comet assay, and global methylation in blood and bone marrow	Not impactful	Unknown test article
Edrissi et al. (2017)	Rat (F344), male Short-term (7, 14, 21, or 28 d; 6 hr/d)	[13C]-labeled formaldehyde 0, 2 ppm Inhalation	FA-lysine adducts in bone marrow and WBCs	Not impactful	Adducts may or may not lead to more robust markers

Reference	Study design	Exposure[a]	Mechanistic endpoints	Impact	Rationale
Ge et al. (2020a)	Mouse (Balb/c), male Short-term (2 wk; 8 hr/d, 5 d/wk)	Formalin 0, 0.5, 3 mg/m^3 Inhalation	Myeloid progenitor cell (BFU-E and CFU-GM) colony counts and cytokines; bone marrow histology, ROS, and gene expression of cell cycle and DNA damage markers	Not impactful	Formalin; short-term (otherwise important endpoints)
Wei et al. (2017b)	Mouse (BALB/c), male Short-term (2 wk; 8 hr/d, 5 d/wk)	Formalin 0, 3 mg/m^3 Inhalation	Bone marrow - myeloid progenitor formation assay, bone marrow cellularity	Not impactful	Formalin; short-term (otherwise important endpoints)
Wei et al. (2017a)	Mouse (BALB/c), male, Short-term (2 wk; 5 d/wk), followed by 7 d recovery	Formalin 0, 3 mg/m^3 Inhalation	Complete blood count, bone marrow histopathology, myeloid progenitor colony-forming cell assay, ROS and inflammatory markers, DNA-protein crosslinks	Not impactful	Formalin; short-term (otherwise important endpoints)
Zhao et al. (2020)	Mouse (Balb/c), male Short-term (2 wk; 8 hr/d, 5 d/wk) (note: ex vivo systemic tissues not PECO relevant)	Formalin 0, 3 mg/m^3	Formation of burst-forming unit-erythroid (BFU-E), and colony-forming unit-granulocyte macrophage (CFU-GM) cellular colonies in bone marrow and spleen	Not impactful	Formalin; short-term (otherwise important endpoints)
Modeling, Endogenous Formaldehyde, and Other Studies					
Burgos-Barragan et al. (2017)	Mouse (C57BL/6 × 129SV hybrid background), WT or KO in ALDH2, FANCD2, or both (note: also includes in vitro evaluations in human, chicken, and mouse cells)	No formaldehyde inhalation exposures (note: included since it evaluates essentiality of formaldehyde detoxification in normal processes)	Colony Forming Units (CFU) from bone marrow stem cells and progenitor cells	Possibly impactful	Serves as included reference study for discussion of potential sources of susceptibility relating to formaldehyde detoxification; cell production from bone marrow is an important endpoint
Dingler et al. (2020)	Mouse (C57BL/6 background), ALDH2 and ALDH5 WT, single, and double KO, both sexes (note: also includes	No formaldehyde inhalation exposures (note: included since it evaluates essentiality of formaldehyde	Genotoxicity in peripheral blood cells and bone marrow (MN assay, SCE); bone marrow stem cell and progenitor cell quantification, lineage characterization, and B	Possibly impactful	Serves as included reference study for discussion of potential sources of susceptibility relating to formaldehyde

Reference	Study design	Exposure[a]	Mechanistic endpoints	Impact	Rationale
	primary cultures of human PBLs, fibroblasts, and buccal cells not deemed PECO-relevant)	detoxification processes in normal function)	cell maturation; thymic development and cell lineage characterization; complete blood cell count, cell cycle profiling		detoxification; hematopoietic health and cell production from bone marrow is important endpoint
García-Calderón et al. (2018)	Mouse (C57BL/6 background), WT or KO in ALDH5 or FANCD2 (note: also includes in vitro evaluations not deemed PECO-relevant)	No formaldehyde inhalation exposures (note: included since it evaluates essentiality of formaldehyde detoxification in normal processes)	Bone marrow HSPC lineage, function, and genotoxicity; complete blood cell count	Possibly impactful	Serves as included reference study for discussion of potential sources of susceptibility relating to formaldehyde detoxification; hematopoietic health and cell production from bone marrow are important endpoints
Nakamura et al. (2020)	Mouse (C57BL/6 background), ALDH2 and ALDH5 WT, single, and double KO, both sexes Observed GD0 to PND25	No formaldehyde inhalation exposures (note: included since it evaluates essentiality of formaldehyde detoxification processes in normal function)	Postnatal survival and gross organ observations (e.g., spleen, liver, lung thymus)	Not impactful	Serves as included reference study for discussion of potential sources of susceptibility relating to formaldehyde detoxification
Starr and Swenberg (2016)	Update to prior non-primary research perspectives on how to calculate cancer risk			Not impactful	Included here because commented on in existing draft, but non-primary research

Abbreviations: PBL = peripheral blood leukocytes; MN = micronucleus; WBC = white blood cell.
Rows for studies judged as "not impactful" are shaded grey; unshaded rows highlight studies incorporated into the updated draft assessment.
[a] Use of methanol-stabilized formalin was inferred in some studies based on study-specific description (e.g., 37% stock solution).

F.3.12. Nervous System Effects

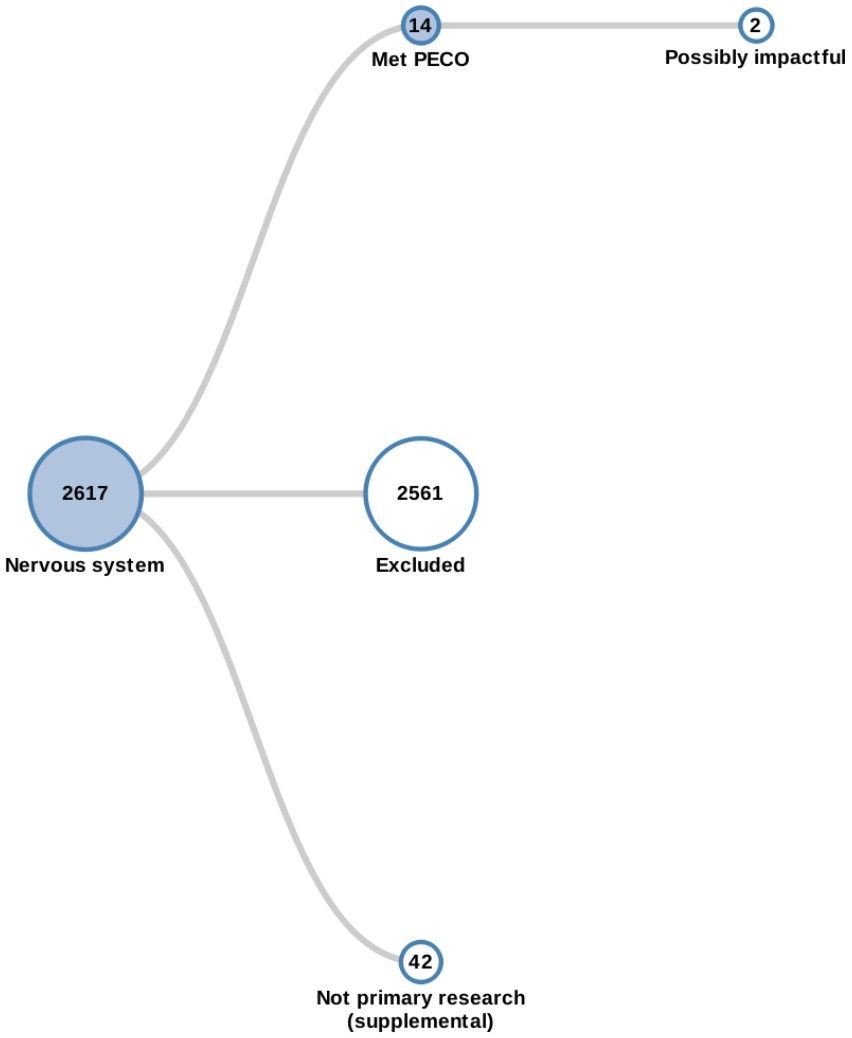


Figure F-12. Nervous system effects literature tree (interactive version here).

A total of 2,617 citations were retrieved for the assessment of nervous system effects and 14 studies were PECO-relevant (Table F-14). Of these, two human studies were deemed to be possibly impactful. Peters et al. (2017) was identified in the literature search update and included in the inventory table although it already had been included in the 2017 draft Toxicological Review of Formaldehyde-Inhalation. None of the identified animal or mechanistic studies were deemed possibly impactful.

Table F-14. Studies of nervous system effects

Reference	Study design	Exposure[a]	Endpoints	Impact	Rationale
Human Studies					
Bellavia et al. (2021)[b]	General population Denmark case-control	Occupational history and job-exposure matrix, adults	Amyotrophic lateral sclerosis (ALS)	Possibly impactful	Additional study on health effect for which there are few studies
Peters et al. (2017)	General population Sweden case-control	Occupational history and job-exposure matrix, adults	Amyotrophic lateral sclerosis (ALS) incidence	Possibly impactful	Already identified in 2017 draft
Animal Studies[c]					
Askar and Halloull (2018)	Rat (Albino, strain not specified), male Subchronic (12 wk; 6 hr/d, 5 d/wk)	Paraformaldehyde 0, 20 ppm (0, 24.6 mg/m^3) Inhalation	Cerebellar histopathology, cell counts, and cell morphology; evaluations of ROS and inflammatory markers	Not impactful	High levels
Cheng et al. (2016)	Mouse (Kunming), male Short-term (Up to 7 d; continuous)	Formalin 0, 0.08, 0.8 mg/m^3 Inhalation	Morris water maze	Not impactful	Formalin
Duan et al. (2018)	Mouse (Balb/c), male Short-term (18 d; 5 hr/d)	Formalin 0, 1 mg/m^3 Inhalation	Prefrontal cortex histology; brain ROS and inflammation markers, cytokines	Not impactful	Formalin; no saline plus formaldehyde control group
Ge et al. (2019)	Mouse (Kunming), male Short-term (21 d; continuous)	Formalin 0, 1 mg/m^3 Inhalation	Morris water maze, hippocampal morphology, brain ROS and cell signaling markers	Not impactful	Formalin
Huang et al. (2019)	Mouse (Kunming), male Short-term (14 d; 8 hr/d)	Formalin 0, 3 mg/m^3 Inhalation	Morris water maze; brain ROS and inflammatory markers; hippocampal histopathology and cell morphology	Not impactful	Formalin
Li et al. (2016)	Mouse (Kunming), male Short-term (7 d; 2 hr/d)	Formalin 0, 1, 2 ppm (0, 1.23, 2.46 mg/m^3) Inhalation	Open field activity; elevated plus maze test; forced swimming test; novel object recognition; counts of TH- and GR-immunoreactive neurons	Not impactful	Formalin; brief exposures

Reference	Study design	Exposure[a]	Endpoints	Impact	Rationale
Li et al. (2020)	Mouse (Kunming), male Short-term (14 d; 8 hr/d)	Formalin 0, 0.5, 3 mg/m^3 Inhalation	Morris water maze; brain ROS and inflammatory markers; hippocampal histopathology and cell morphology	Not impactful	Formalin
	Mouse (Balb/c), male Short-term (7 d; 8 hr/d)		Brain neurotransmitters; ROS and inflammatory markers in hippocampus, brain stem, and cerebral cortex		
Mei et al. (2016)	Mouse (Balb/c), male Short-term (7 d; 8 hr/d) (in vitro experiments not PECO-relevant)	Unspecified test article 0, 3 mg/m^3 Inhalation	Morris water maze; qualitative hippocampal neuron staining; brain ROS and GSH	Not impactful	Formalin
Zhang et al. (2014b)	Rat (Sprague Dawley), male Short-term (14 d; 30-min, 2×/d)	Unspecified test article 0, 13.5 ppm (0, 16.6 mg/m^3) Inhalation	Buried food pellet behavioral testing; olfactory bulb synaptosomal and neuronal markers; olfactory sensory neuron maturation	Not impactful	Unknown test article; high levels; brief exposures
Mechanistic Studies					
Cao et al. (2015)	Mouse (Balb/c), male Short-term (7 d; 8 h/d)	Unspecified test article 0, 0.5, 3 mg/m^3 Inhalation	Hippocampus, cortex, and brainstem ROS and inflammatory markers	Not impactful	Unknown test article
Eom et al. (2017)	*Drosophila melanogaster* (mutant strains: WT, p53 and p38b) Acute (6 or 24 hr)	Unspecified test article 0, 10, 100 µg/m^3 Inhalation	Behavioral (movement-based) quantification; microarray analyses (note survival test study design not extracted)	Not impactful	Non-mammalian; unknown test article
Li et al. (2015)	mouse (ICR), male, Acute or short-term (1 or 7 d; 6 hr/d)	Unspecified test article 0, 3 ppm (0, 3.69 mg/m^3) Inhalation	miRNA screening of olfactory bulb	Not impactful	Unknown test article

Rows for studies judged as "not impactful" are shaded grey; unshaded rows highlight studies incorporated into the updated draft assessment.

[a]Use of methanol-stabilized formalin was inferred in some studies based on study-specific description (e.g., 37% stock solution).

[b]An additional study, Seals et al.(2017), was identified from the reference list of Bellavia et al. (2021). As this study was determined to be possibly impactful to the 2017 draft conclusions on nervous system effects, it was incorporated into the Toxicological Review.

[c]Animal studies may include evaluation of mechanistic endpoints.

F.3.13. Reproductive and Developmental Effects

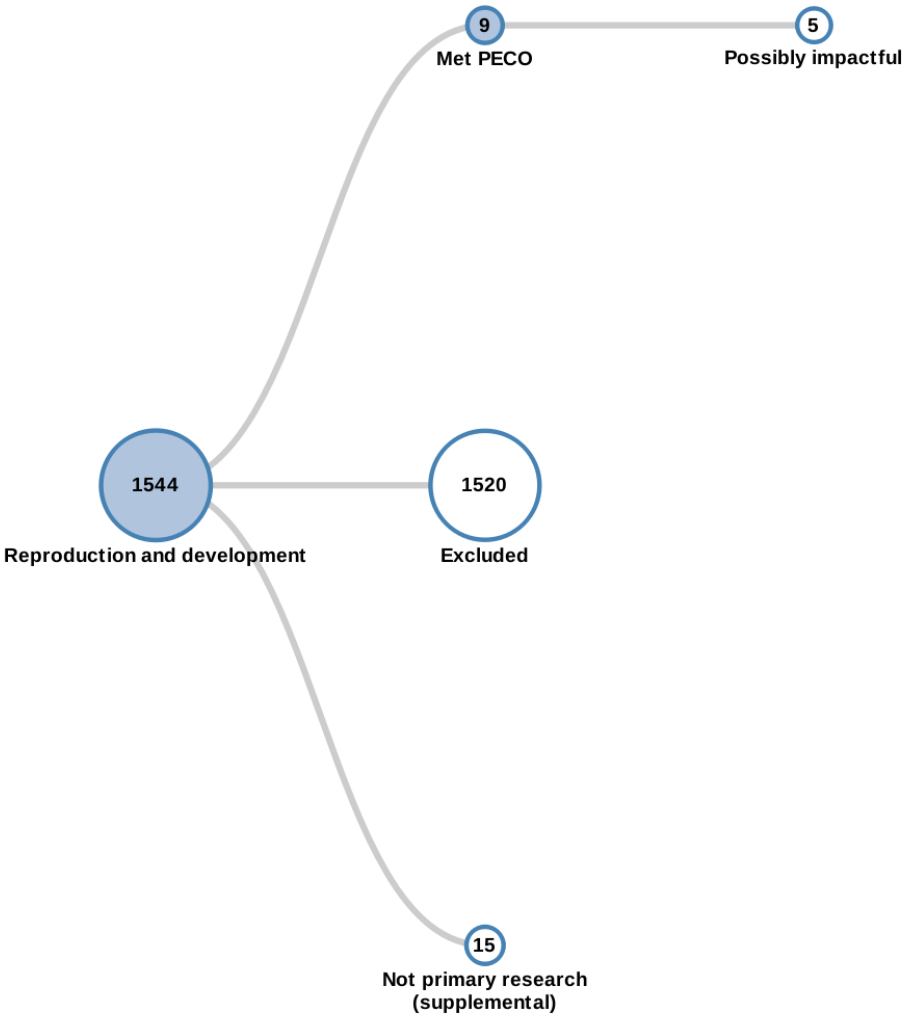


Figure F-13. Reproductive and developmental effects literature tree
(interactive version here).

A total of 1,544 citations were retrieved for the assessment of reproductive and developmental effects and 9 studies were PECO-relevant (Table F-15). Of these, five were deemed to be possibly impactful. There were four from the human literature and one from the animal literature. Neither of the identified mechanistic studies were deemed possibly impactful. Wang et al. (2015) was identified in the literature search update and included in the inventory table although it already had been included in the 2017 draft Toxicological Review of Formaldehyde-Inhalation.

Table F-15. Studies of reproductive and developmental effects

Reference	Study design	Exposure[a]	Endpoints	Impact	Rationale
Human Studies					
Amiri and Turner-Henson (2017)	General population southeastern U.S. cross-sectional	Air sampling, prenatal, exposure during pregnancy	Biparietal diameter, head circumference, abdominal circumference, femur length, ratio of abdominal circumference to femur length (AC/FL), estimated fetal weight	Possibly impactful	Health effect for which there are few studies
Chang et al. (2017)	General population Seoul, South Korea birth cohort	Air sampling, prenatal, exposure during pregnancy	Birthweight, postnatal weight at 6, 12, 24, and 36 mos	Possibly impactful	Health effect for which there are few studies
Franklin et al. (2019)	General population Australia birth cohort	Air sampling, prenatal, exposure during pregnancy	Gestational age, birth length, birth weight, head circumference	Possibly impactful	Health effect for which there are few studies
Wang et al. (2015)	Occupational China cross-sectional	Air sampling and occupational history, adults, male plywood production workers	Semen volume, sperm concentration, total sperm count, sperm progressive motility and total sperm motility, curvilinear velocity, straight line velocity, linearity, time-average velocity, straightness, mean angular displacement, amplitude of lateral head displacement	Possibly impactful	Already identified in 2017 draft
Animal Studies[b]					
Sapmaz et al. (2018)	Rat (Sprague Dawley), male Short-term (4 wk) or Subchronic (13 wk), 8 hr/d, 5 d/wk	Paraformaldehyde 0, 5 ppm (0, 6.15 mg/m^3) Inhalation	Testicular tubular atrophy, germinative epithelium height, seminiferous tubule diameter; markers of ROS in testicular tissue	Possibly impactful	Longer duration study; informative morphological endpoints
Ge et al. (2020b)	Rat (Sprague Dawley), male Subchronic (8 wk)	Formalin 0, 0.5, 2.46, 5 mg/m^3 Inhalation	Testicular seminiferous tubule histopathology and morphometry, SPO11 protein in testicular tissue	Not impactful	Formalin
Zang et al. (2017)	Mouse (C57BL/6), male	Formalin 0, 0.5, 5, 10 mg/m^3	Sexual behavior (mount latency, intromission latency, ejaculation latency, mount frequency,	Not impactful	Formalin

Reference	Study design	Exposure[a]	Endpoints	Impact	Rationale
	Subchronic (60 d; 4 hr/d)	Inhalation	intromission frequency, copulatory efficacy); hormone measures (serum T and LH; testicular T); sperm number and motility; reproductive organ weights and histopathology		
Mechanistic Studies					
Fang et al. (2015)	Rat (Sprague Dawley), male Short-term (4 wk; 8 hr/d)	Unspecified test article 0, 0.5, 5, 10 mg/m^3 Inhalation	mTOR (mammalian target of rapamycin, a regulator of various cellular processes) mRNA expression, protein levels, and immunostaining in testes	Not impactful	Unspecified test article
Ibrahim et al. (2016)	Rat (Wistar), female (dam) Gestational (GD1-21; 1 hr/d, 5 d/wk)	Unspecified test article 0, 0.92 mg/m^3 Inhalation	Markers of ROS and inflammation in dam uterus at parturition; inflammation and immune parameters in offspring after PND30: BAL cell count and myeloperoxidase activity, lung cytokines and inflammatory markers; blood and bone marrow cell counts	Not impactful	Unspecified test article

Rows for studies judged as "not impactful" are shaded grey; unshaded rows highlight studies incorporated into the updated draft assessment.
[a]Use of methanol-stabilized formalin was inferred in some studies based on study-specific description (e.g., 37% stock solution).
[b]Animal studies may include evaluation of mechanistic endpoints.

APPENDIX G. QUALITY ASSURANCE FOR THE IRIS TOXICOLOGICAL REVIEW OF FORMALDEHYDE

This assessment is prepared under the auspices of the U.S. Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS) Program. The IRIS Program is housed within the Office of Research and Development (ORD) in the Center for Public Health and Environmental Assessment (CPHEA). EPA has an agency-wide quality assurance (QA) policy that is outlined in the *EPA Quality Manual for Environmental Programs* (see CIO 2105-P-01.1) and follows the specifications outlined in EPA Order CIO 2105.1.

As required by CIO 2105.1, ORD maintains a Quality Management Program, which is documented in an internal Quality Management Plan (QMP). The latest version was developed in 2013 using Guidance for Developing Quality Systems for Environmental Programs (QA/G-1). An NCEA/CPHEA-specific QMP was also developed in 2013 as an appendix to the ORD QMP. Quality assurance for products developed within CPHEA is managed under the ORD QMP and applicable appendices.

The IRIS Toxicological Review of Forrmaldehyde is designated as Highly Influential Scientific Information (HISA)/Influential Scientific Information (ISI) and is classified as QA Category A. Category A designations require reporting of all critical QA activities, including audits. The development of IRIS assessments is done through a seven-step process. Documentation of this process is available on the IRIS website: https://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#process.

Specific management of quality assurance within the IRIS Program is documented in a Programmatic Quality Assurance Project Plan (PQAPP). A PQAPP is developed using the EPA Guidance for Quality Assurance Project Plans (QA/G-5), and the latest approved version is dated April 2021. All IRIS assessments follow the IRIS PQAPP, and all assessment leads and team members are required to receive QA training on the IRIS PQAPP. During assessment development, additional QAPPs may be applied for quality assurance management. They include

Title	Document number	Date
Program Quality Assurance Project Plan (PQAPP) for the Integrated Risk Information System (IRIS) Program	L-CPAD-0030729-QP-1-4	April 2021
An Umbrella Quality Assurance Project Plan (QAPP) for Dosimetry and Mechanism-Based Models (PBPK)	L-CPAD-0032188-QP-1-2	December 2020
Quality Assurance Project Plan (QAPP) for Enhancements to Benchmark Dose Software (BMDS)	L-HEEAD-0032189-QP-1-2	October 2020

During assessment development, this project undergoes one quality audit during assessment development including:

Date	Type of audit	Major findings	Actions taken
July 27, 2021	Technical system audit	None	None

During Step 3 and Step 6 of the IRIS process, the IRIS toxicological review is subjected to external reviews by other federal agency partners, including the Executive Offices of the White House. Comments during these IRIS process steps are available in the docket EPA-HQ-ORD-2010-0396 on http://www.regulations.gov.

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